

CULTURE AND USE OF ALGAE IN SOUTHEAST ASIA

Proceedings of the Symposium
on Culture and Utilization of Algae
in Southeast Asia



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FOREWORD

The idea of holding this Symposium was brought about by the growing interest in the role of algae in the economic life of the peoples of the developing countries in Southeast Asia. The use of algae as food, as raw materials in the production of industrial phycocolloids, and as natural feed for economically important aquaculture species has been highlighted recently in developed countries in Asia and other parts of the world. The need for information on recent developments in this area of concern was the primary consideration in the holding of this Symposium.

Efforts to organize the Symposium started in early 1981 under the auspices of UNESCO through its Regional Headquarters of Network for Microbiology in Southeast Asia in cooperation with the National Institute of Science and Technology Authority. The Aquaculture Department of the Southeast Asian Fisheries Development Center (SEAFDEC) in Tigbauan, Iloilo, hosted the Symposium and provided the secretariat services and funds to support a number of local participants.

Attendance in the Symposium included fifteen full participants from different institutions from seven countries, students and faculty of the University of the Philippines in the Visayas, and other observers.

Thirteen papers were presented covering a wide variety of topics from culture of micro and macroalgae to processing of algal products and their utilization in industry as natural feed for aquaculture animals, status of seaweed resources and their production, and the biology and use of algal population as indicator of the environmental state of the aquatic environment.

This late the Aquaculture Department of SEAFDEC decided to publish the proceedings of the Symposium because the information therein continue to be relevant to aquaculture.

F.J. Lacanilao
Chief
Aquaculture Department
SEAFDEC

February 1990



Participants from Indonesia, Japan, Korea, Malaysia, Philippines, Singapore and Thailand in the *Symposium on Culture and Use of Algae in Southeast Asia*, 8-11 December 1981, Tigbauan Research Station, SEAFDEC Aquaculture Department, Tigbauan, Iloilo, Philippines.

SEAWEED RESOURCES IN THE DEVELOPING COUNTRIES OF ASIA: PRODUCTION AND SOCIO-ECONOMIC IMPLICATIONS

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ABSTRACT

The bulk of world seaweed production today comes from developed countries in the temperate region, including Japan, China, and Korea. The seaweed production potentials in the developing countries of Asia will have to be explored to meet the increasing world demand. Extensive shallow and farmable reef areas as well as cheap labor highly favor seaweed production. Harvesting from natural stocks, usually the practice in most developing countries, is unreliable. Efforts should thus be directed toward actual farming of seaweeds.

In the Philippines, development of the farming technology on *Euचेuma alvarezii* and *E. denticulatum* significantly increased production by the middle of the 70's. Harvests of *Euचेuma* from farms and other seaweeds from natural stocks now rank third among the fishery exports of the country.

The socioeconomic implications of the development of the seaweed resources in the developing countries of Asia are discussed. The Philippine experience is cited specifically to show the benefits derived from seaweed farming technology.

INTRODUCTION

Although no accurate data are available, world production of seaweeds at present seems quite sizeable. For instance, Naylor (1976) estimates a world production of 1 170 000 and 2 400 000 metric tons (mt) of wet weight for 1963 and 1973, respectively. The 1973 production was valued at approximately US\$765 million at primary source. In 1974, production was estimated at 2 664 000 mt. An examination of the data, however, shows that the bulk of production was contributed by developed countries in the temperate

regions including Japan, China, and Korea which produced 697 800 mt (1974), 700 000 mt (1973), and 335 700 mt (1975), respectively. These countries are the major producers of seaweeds in Asia.

In contrast, developing countries contributed very little to the total seaweed production in the world. For instance, among the Asian countries, the Philippines produced only 7 000, Indonesia 15 000 and India 6 000 mt, or an aggregate of only 28 000 mt in 1974. Productions in the other countries of Asia were negligible. Hence the 1974 production of these countries represents a very small portion of the total world production. Naylor's (1976) estimate for the Philippines, however, is incorrect. The 7 000 mt produced in 1974 was on dry weight, not wet weight, basis.

Present estimates (Naylor 1976) show that seaweed production in developed countries cannot cope with the highly increasing demands for food, pharmaceutical, and industrial purposes. It is estimated that the annual increase in the demand for seaweeds and seaweed products is 8-10% (Naylor 1976). Hence, countries such as the United States, Canada, Denmark, Japan, France, Australia, and Germany are importing large amounts of dried seaweeds from developing countries in Southeast Asia.

STATUS OF SEAWEED PRODUCTION IN ASIA

Except for Japan, China, and Korea which have a well-established seaweed industry, most Asian countries are still dependent on the wild or natural seaweed crops, i.e., fishermen directly gather seaweeds from natural stocks. Actual farming of seaweeds in the Philippines is limited only to three commercial species, e.g., *Eucheuma alvarezii* (commercially known as the "cottonii type"), *E. denticulatum* (the "spinosum type") and *Caulerpa lentillifera*. The first two species are produced through mariculture while *C. lentillifera* is cultivated in ponds.

The harvesting of natural seaweed stocks is very unreliable. Production is highly dependent on the growth of the species which in turn is highly influenced by monsoons and other environmental stresses. In addition, the absence of a management program for the naturally produced species often results in the depletion and/or destruction of natural stocks due to over-harvesting. In contrast, production through farming is not only reliable but is also a very efficient way of conserving local stocks. The principal seaweed genera, their uses, and present status of production in Asia are listed in Table 1.

The potentials of seaweed production in the developing countries of Asia are very high. Many Asian countries possess well developed coral reefs which could support high seaweed production. These shallow and farmable reef

areas, however, are presently utilized mainly for small-scale fishing. In the Philippines, the increased harvests of *Eucheuma* from farms reflect the development of such potentials and have made the country the major supplier of this species in the international market. Due to low production costs, seaweeds and seaweed products from developing countries in Asia can be highly competitive in the international market.

Table 1. Principal seaweed genera of economic potential in Asia

Country	Genera	Uses	Status of production
Philippines	<i>Caulerpa</i>	food	pond culture & wild crops
	<i>Codium</i>	food	wild crops
	<i>Sargassum</i>	alginate, feeds	wild crops
	<i>Porphyra</i>	food	wild crops
	<i>Gelidiella</i>	agar	wild crops
	<i>Gracilaria</i>	agar, food	wild crops
	<i>Eucheuma</i>	carrageenan, food	mariculture
Indonesia	<i>Gracilaria</i>	agar, food	wild crops
	<i>Eucheuma</i>	carrageenan	wild crops, mariculture
	<i>Gelidiella</i>	agar, food	wild crops
	<i>Hypnea</i>	carrageenan, food	wild crops
	<i>Caulerpa</i>	food	wild crops
	<i>Acanthophora</i>	food	wild crops
Singapore	<i>Eucheuma</i>	carrageenan	wild crops
	<i>Gracilaria</i>	agar	wild crops
	<i>Sargassum</i>	alginate	wild crops
Brunei	<i>Gracilaria</i>	agar	wild crops
East Malaysia	<i>Porphyra</i>	food	wild crops
	<i>Sargassum</i>	alginate	wild crops
	<i>Eucheuma</i>	carrageenan	wild crops
	<i>Caulerpa</i>	food	wild crops
	<i>Gracilaria</i>	agar	wild crops
West Malaysia	<i>Gracilaria</i>	agar	wild crops
Thailand	<i>Gracilaria</i>	agar	wild crops
	<i>Porphyra</i>	food	wild crops
Vietnam	<i>Gracilaria</i>	agar	wild crops
	<i>Sargassum</i>	alginate	wild crops
Hong Kong	<i>Sargassum</i>	alginate	wild crops
	<i>Porphyra</i>	food	wild crops

Table 1 continued..

Taiwan	<i>Gracilaria</i> <i>Porphyra</i>	agar food	pond culture wild crops; culture
Sri-Lanka	<i>Gracilaria</i> <i>Porphyra</i>	agar food	wild crops wild crops
India	<i>Gracilaria</i> <i>Gelidiella</i> <i>Sargassum</i> <i>Hypnea</i>	agar agar alginate carrageenan	wild crops; mariculture wild crops wild crops wild crops
Burma	<i>Gracilaria</i> <i>Gelidium</i> <i>Sargassum</i>	agar agar alginate	wild crops wild crops wild crops
Pakistan	<i>Gracilaria</i> <i>Gelidium</i> <i>Hypnea</i> <i>Porphyra</i>	agar agar carrageenan food	wild crops wild crops wild crops wild crops

SEAWEED PRODUCTION IN THE PHILIPPINES

The Philippine seaweed export profile is reflected in Table 2. No official records of local seaweed production are available before 1967 when the Philippines started exporting dried seaweeds to other countries. Production from 1967 to about 1972 was mostly harvests from natural stocks by fishermen. During this period, the unregulated harvests in response to the high demand of the dried produce in the world market led to the depletion of the natural stocks. Toward the end of the 1960's and during the early 1970's, production was maintained mainly by the discovery of new seaweed beds in very far and hardly accessible reef areas.

The development of farming technology in the early 70's made its full impact on production toward the middle 70's when production came mainly from the farming of two species of *Eucheuma*, namely, *E. alvarezii* and *E. denticulatum*. The farming of the second species contributed significantly to production during the latter half of the decade. A small portion of the total seaweed production was derived from natural stocks of other seaweeds such as *Gracilaria*, *Gelidiella*, *Caulerpa*, and *Sargassum*. Except for *Caulerpa*, their production is dependent up to now on natural stocks.

Pond culture of *Caulerpa* is presently done in Mactan, Cebu. Although the produce is locally sold in open markets of Metro Manila, Cebu City, Cagayan de Oro City, and Zamboanga City, recently a significant portion is being exported to Okinawa in a partially dehydrated (salted) state.

Table 2. Philippine seaweed export, 1967-1980

Year	Metric Ton	Value (Pesos)
1967	674.5	351 989
1968	263.9	221 056
1969	427.6	447 908
1970	318.1	527 321
1971	339.8	675 504
1972	483.9	1 414 051
1973	1 432.7	4 062 086
1974	5 039.6	14 973 151
1975	4 514.8	13 292 226
1976	3 950.1	12 366 568
1977	6 094.1	14 666 768
1978	13 575.3	42 480 674
1979	16 495.8	58 521 274
1980	13 191.3	55 667 616

Source: Exports of fish and fishery products cleared by the Bureau of Fisheries and Aquatic Resources, by kind, quantity, and value, Fisheries Statistics, 1967-1980.

All *Sargassum* productions in Central Visayas are being exported to Japan whereas those in Northern Mindanao are utilized in the local manufacture of feeds. A significant amount of *Gracilaria* and *Gelidiella* is exported while the rest is locally processed into crude agar bars. Production of other genera such as *Codium*, *Hypnea*, and *Porphyra* is dependent on natural stocks and the produce is only locally consumed (BFAR Statistics 1980).

The data on Philippine seaweed exports indicate an almost twenty-fold increase in production from 1967 to 1980 (BFAR Statistics 1980). From a minor product, seaweeds now rank third, behind tuna and shrimps, among Philippine fishery exports.

There are many other seaweed species which still remain to be tapped and/or developed. The rational development of these resources, however, is hampered by the lack of appreciation on the part of the policy makers of the importance and economic potentials of seaweeds as a fishery resource. This attitude is reflected in the priorities and the funding support allocated to seaweed research and development compared to other natural resources such as energy, forestry, minerals, etc. Notwithstanding the many reasons for this negative attitude, the developing countries of Asia should take a hard look at the history of the seaweed industry of Japan, China, and Korea where seaweeds as a resource touch on the everyday life of the people. Once this attitude is resolved, the solution to the other major problems such as lack of expertise, facilities, and funds will naturally follow. Solving these problems may take some time but surely time will be a minor constraint. And the resolution of the foremost problem of lack of appreciation for seaweeds may

be hastened through the influence of international agencies such as the UNESCO, FAO, UNDP, and others.

SOCIOECONOMIC IMPLICATIONS OF SEAWEED RESOURCE DEVELOPMENT

The socioeconomic impact of the development of the seaweed resources in the developing countries can be readily appreciated by first looking at the present status of the quality of life, resources, and livelihood of the people living in coastal areas in relation to the present emphasis on industrial development and urbanization.

A large portion of the Asian population lives along coastal areas and is intimately associated with the sea and its resources. Most of these coastal populations are located in far-flung areas which are hardly benefited by modern industrial development and urbanization. Being in the tropics, the coastal areas of Asian countries are characterized by well-developed coral reefs, shallow bays and coves which used to abound with fishery resources. Through intensive fishing, these coastal areas have been and will continue to be depleted of resources on which the very lives of the coastal population directly or indirectly depend. The grave concern for the consequences of the depletion of the fisheries in shallow coastal areas is best dramatized by the closure of traditional fishing grounds to big fishing operations. The Philippines, Indonesia and Thailand have enacted measures to prevent the over-exploitation of the fishery stocks in some fishing grounds in consideration of the plight of the small-scale or artisanal fishermen who can hardly afford to have more sophisticated fishing crafts to fish in far areas which still support good fisheries. The decline in coastal productivity coupled with population increase consequently would lead to the lowering of living standards in the coastal areas. The development of seaweeds as a resource is an alternative activity which should rank high in government programs if the idea of a more equitable distribution of wealth and benefit is to accommodate the poor fishermen. In the Philippines, for instance, more than 600 000 of the fishing force are small-scale fishermen.

A Philippine experience, as exemplified by the development of the Danajon Reef in northern Bohol in Central Visayas, exquisitely demonstrates the socioeconomic benefits that can be derived from seaweed farming. The development of *Eucheuma* farming in this area is better documented than that in the Sulu Archipelago. During the peak production of *E. denticulatum* (Lim and Porse 1980) in northern Bohol in 1979 there were: 200 farm houses and drying platforms constructed, 500 hectares of the reef planted with seaweeds, 2 000 people working daily on the farms, and 1 000 families or approximately 8 500 people fully or partially dependent on seaweed farming.

The above data do not include the people who were in some way benefited by the farming activities, e.g., the middlemen, the small store owners, and suppliers of farm materials, among others. The secondary impact of the farming activities is of course very hard to measure. However, favorable effect which the seaweeds brought to the people in terms of improvement of their life styles was best illustrated by their acquisition of simple luxury items such as radios, gas stoves, clothes, and motorized bancas. But the best proof that seaweed farming is a productive form of livelihood is the shift from fishing into seaweed farming. Results of interviews show that local fishermen were earning a net average of P12 per day. A hectare of seaweed farm netted a farmer an average of P1 200 per month excluding the capital investment.

The production in northern Bohol in 1979 was, however, small compared to that in Sulu in the southern Philippines which still accounts for the bulk of farmed seaweed.

As an entrepreneur, the seaweed farmer is subject to both favorable and adverse effects of the demand and price fluctuations in the international market.

Since seaweed farming is labor-intensive, the industry can employ the otherwise idle or underutilized labor force in the coastal areas.

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UTILIZATION AND FARMING OF SEAWEEDS IN INDONESIA

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ABSTRACT

A great variety of seaweeds grow abundantly along the 81 000-km coastline of the 13 000 islands comprising the Indonesian archipelago. However, it is only recently that the economic importance of seaweeds has really been appreciated.

At present, seaweeds collected in Indonesia are mainly used for food supplement, domestic agar manufacture, and for export. Because of the increasing demands for the carrageenan-containing seaweed, mass cultures have been undertaken in both experimental and production sites established in many parts of the country. These efforts are expected to increase the annual volume of exports from 2 000 to 6 000 mt.

The paper reviews the state and problems of seaweed utilization, development, and farming efforts in Indonesia.

INTRODUCTION

The Indonesian archipelago is situated between the Asian and the Australian continents and between the Pacific and the Indian Oceans. Geographically, it is located between 94°-141°E and 6°N-11°S. Roughly, it consists of 13 667 islands with more than 81 000 km of coastline. Marine waters constituting two-thirds of the country's territory are an ample resource for maritime opportunities. Hence, a considerable amount of scientific survey and research on the marine environment had been undertaken.

Early marine research in Indonesia started when Amboina was a headquarter for the Dutch East Indies Company. However, modern marine research stemmed from the Siboga Expedition of 1899-1900 (Tydeman 1903) which focused on the marine flora and fauna and their biogeography. Physical oceanography was brought to the fore in the 1930's by the Snellius

(Riehl 1952) and the Dana Expeditions and more recently by the world-renowned Albatross and Galathea Expeditions in 1948 and 1951, respectively.

The earliest report on seaweeds in Indonesian waters probably was that of Rumphius (1750). Through the untiring effort of Weber van Bosse during the Siboga Expedition, phycology flourished in Indonesia. Unfortunately, however, it is only recently that the economic potential of seaweeds has been appreciated. This paper describes the state of research on algae and their significance in Indonesia.

SEAWEED PRODUCTION

A number of surveys indicate that the distribution and density of marine algae in different areas vary according to the type of bottom, season, hydrographic conditions, and species composition at a given time. Table 1 presents the great variations in standing crop from one region to another and from one species to another. It is, thus, imperative to consider these differences in the utilization of seaweed resources.

Table 1. Variations in standing crops of seaweed in Indonesia and other countries

Location	Species	Standing crop (tons/ha)
INDONESIA		
Wawarada Bay	<i>Euclima spinosum</i>	4-18**
Moluccas and East Nusa Tenggara	<i>E. spinosum</i>	0.6-3.4**
Seribu Islands	<i>E. spinosum</i>	0.11**
Tanjung Benoa	<i>E. serra</i>	0.46**
	<i>Gracilaria lichenoides</i>	0.96**
	<i>Hypnea</i> spp.	1.52**
	<i>Ulva</i> spp.	1.63**
Southeast Moluccas	<i>E. spinosum</i>	2.27**
Central Moluccas	<i>E. edule</i>	5.02**
	<i>Gracilaria</i> spp.	2.13**
OTHERS		
Philippines	<i>E. spinosum</i>	9**
La Jolla, California	<i>Laminaria</i> sp.	62.5-100**
Scotland	<i>Laminaria cloustonii</i>	19.5-45**
Hanauma Bay,	<i>Sargassum obtusifolium</i>	5.7-10.2*
Hawaii	<i>S. echinocarpum</i>	4.7-6.5**
Kaneohe Bay, Hawaii	<i>Dictyosphaeria cavernosa</i>	0.07-7.27*

* Dry weight; ** Wet weight.

The identification of factors responsible for such variations must then be one major area for seaweed research. Fortunately, this has been recognized in Indonesia recently. For example, Soegiarto (1963) observed that the standing crop of seaweeds in Wawarada Bay, Sumbawa, ranged from 4 to 18 tons wet weight per hectare and about half (52.6%) of the mixed populations consisted of *Euचेuma spinosum*. In 1974, a large-scale survey was carried out to determine the location and production potentials of the seaweed *Euचेuma* in the Moluccas and the East Nusa Tenggara (Lesser Sunda) waters (Mubarak 1974). Based on this survey, it was estimated that production of *Euचेuma* from the Moluccas is about 1 750 mt/yr and from the East Nusa Tenggara 200 mt/yr. It seems that these estimates were rather low since figures from the Directorate General of Fisheries showed that the Moluccas produced 2 277 mt in 1973, 2 636 mt in 1974 and 7 160 mt in 1975 (Table 2). This discrepancy only indicates that further surveys and research are needed in order to arrive at reasonable estimates on seaweed production in each region. Other surveys were carried out by Sulustijo and Atmadja (1980) in the Seribu Islands, Jakarta Bay; Sulustijo and Yusuf in Southeast Moluccas; and Sahupala et al. (1977) in Central Moluccas.

UTILIZATION OF SEaweEDS

At present, the seaweeds collected in Indonesia are mainly used for food, agar extraction, and export.

Table 2. Seaweed production from selected coastal waters of Indonesia*

Place	Production (metric tons)		
	1973	1974	1975
Bengkulu, West Sumatra	5	5	3
South Sumatra	39	13	645
Riau Archipelago (Strait of Malacca)	**	**	195
Northcoast of East Java	56	234	**
Yogyakarta, Southcoast of Java	**	**	9
Bali	60	74	61
Lesser Sunda Islands (Nusa Tenggara)	329	154	150
Southeast Celebes	387	126	203
Moluccas	2 277	2 636	7 160
West Irian	3	990	**

*Source: The Indonesian Directorate General of Fisheries.

**No available data.

Food

The widespread use of seaweeds as human food in Indonesia was recorded as early as 1292 when the first European ships sailed through Indonesian waters. For centuries, the islanders utilized marine algae as food supplement, especially as "sayur" or vegetable (Zaneveld 1955). However, algal consumption was limited to fishermen and normally these edible species did not reach local markets. But some species used in making sweetened jellies were transported farther inland.

Hogue (1922) mentioned some twenty-one species of useful seaweeds in Indonesia. The list was later expanded by Zaneveld (1955) to include also the economically important algae of the Southeast Asian waters. This fact indicates that Indonesians eat seaweeds in various forms: raw as salads, boiled as vegetable, mixed with various spices, pickled, cooked with coconut milk, for soup thickening, pudding, and sweetened jellies. Some species even serve as medicine.

The organic composition of seaweeds varies from one division to another and from one species to another. For any one species, it can also vary geographically, according to depth, and even from one part of a thallus to another. Therefore, it is hardly surprising that the published data on their organic contents are highly variable.

Table 3 shows the chemical analyses of six red algae collected from Sumbawa Island (Nusa Tenggara). Generally, proteins are present in small quantities and are hardly assimilated by human beings. However, animals may utilize certain proteins better. This is probably one of the reasons for using some seaweeds for stock feed instead of for human consumption.

Carbohydrates, occurring mainly as cell wall and intracellular storage materials, constitute the bulk of algal organic matter. However, the high carbohydrate content is of low value as a source of energy since most of these substances are hardly digested in man. It has been suggested, however, that people who eat seaweeds from childhood may acquire a specialized bacterial flora in the intestines to help digest these algal carbohydrates. Thus, islanders might obtain more food value from seaweeds than others and, possibly also, animals not used to a diet which includes algae.

Even if all the carbohydrates and proteins in seaweeds were fully digestible, these could not significantly supplement the nutrients derived from present food or dietary sources. Rather, the nutritive value of seaweeds is based on their mineral and vitamin contents. Biochemists have definitely established the presence of carotene, and vitamins B₁, B₂, B₁₂, C, D, and E in marine algae. Again, the vitamin concentration varies from one species to another. Intraspecific differences also occur geographically and even with depth.

Table 3. Proximate analysis of some Indonesian seaweeds (After Soegiarto 1968)

Proximate analysis (% dry weight)	<i>Eucheuma spinosum</i>	<i>Eucheuma sp.</i>	<i>Gracilaria sp.</i>	<i>Gracilaria confervoides</i>	<i>Gelidiopsis sp.</i>	<i>Hypnea sp.</i>
Moisture	27.50	16.99	19.01	24.91	12.95	25.15
Protein	5.40	2.48	4.17	3.14	9.98	1.59
Carbohydrates	33.22	63.19	42.59	37.52	54.43	32.25
Fats	8.62	4.30	9.54	5.52	11.09	5.81
Crude fiber	3.01	-	10.51	9.14	-	11.43
Ash	22.25	23.04	14.18	15.77	11.75	23.77

Organic Substances

Seaweeds are important sources of chemical substances for industrial or medical purposes, ranking them among the important resources of the sea. The agar industry in Indonesia is of recent development. Incidentally, the term 'agar' is of Indonesian origin. An attempt to manufacture agar commercially was first reported in 1910. Hofstede (1921a, b, 1923) surveyed the agar content of various seaweeds to determine the possibility of establishing large-scale agar production in Indonesia. But due to rather crude and inadequate analytical methods, he concluded that (1) among the edible seaweeds in Indonesia, only a few contain agar, (2) the agar-containing seaweeds are not abundant, and (3) the seaweeds exported in large quantities do not contain agar. Later, the use of improved methods of analysis, however, showed that the seaweeds occurring in Indonesia contain sufficient agar to justify large-scale agar manufacture for export, an opinion shared also by Zaneveld (1955).

The Indonesian government had been giving full support to the establishment of agar factories. Between 1947 and 1952, the Laboratory for Chemical Research in Bogor analyzed the agar content of practically all species of commercial seaweeds collected from Indonesian waters. This work later showed that *Eucheuma* spp. did not contain agar but another substance known as the iota-carrageen in extract from *E. spinosum* which is becoming more important as an additive in various industrial products, especially in America and Europe.

The first large-scale agar factory in Indonesia was set up in 1930 in Kudus along the north coast of Central Java. More factories were soon established in other cities. Unfortunately, the industry suffered a severe setback during the Second World War. But the high price of agar and full government support after the war stimulated the emergence of smaller factories in Jakarta, Surabaya, Padang, Bandung, and other cities. Table 4 shows the present locations of agar factories in Java.

At present, *Gracilaria lichenoides* is the most important raw material for agar manufacture in Indonesia (Table 4). Two other species also commonly used by the industry are *Gracilaria blodgettii* and *Gelidium latifolium*. On the other hand, *Gracilaria confervoides* is not used in agar processing in Indonesia but is exported from Ujungpandang (Macassar) in rather great quantities. There is, however, a need to improve the quality of the agar product. For example, by using a combination of *G. latifolium* and *G. lichenoides*, an agar of better quality can be obtained. Research in this field is badly needed.

Export

For many years, Indonesian export of seaweeds has been an important

Table 4. Some agar factories in Java (After Soegiarto et al., 1975)

Name	No. of workers	Species used	Raw materials needed (kg/month)	Production (kg/month)
Universal Surabaya	4	<i>Gracilaria</i> spp.	710	42
Sinar Kencana Wonocolo, Surabaya	32	<i>Gracilaria</i> spp. and <i>Gelidium</i> spp.	13 000	800
Sriti Surabaya	-	-	250	15
Sari Jaya Surabaya	-	-	2278	166
Oen Brothers Surabaya	7	<i>Gracilaria</i> spp. and <i>Gelidium</i> spp.	417	25
Sumber Laut Surabaya	7	<i>Gracilaria</i> spp. and <i>Gelidium</i> spp.	1000	60
Hasalin Jakarta	60	<i>Gracilaria</i> spp. and <i>Gelidium</i> spp.	20 000-30 000	2000-3000
Djawa Jakarta	25	<i>Gracilaria</i> spp.	15 000	1500

economic activity. Early records show that for over a century, seaweeds had been exported to China. Before the Second World War, the volume of export was more than 1000 mt/yr but it decreased immediately after the war. In the last few years, the demand has increased considerably. With attractive export regulations on soft products, including many marine resources, the volume of seaweed export reached an all-time high of 5 923 mt in 1966 (Table 5) but it later declined due to changes in export regulations, the government's tight money policy, and the decrease in seaweed price.

Table 5. Annual export of seaweeds (dry weight, metric tons) from major Indonesian ports in 1960-1976

Year	Tanjung Priok, Jakarta	Surabaya	Ujungpandang (Macassar)	Ambon	Others
1960	104.50	197.87	305.20	-	20.00
1961	286.05	72.61	562.99	-	-
1962	76.50	40.76	300.99	-	-
1963	146.00	-	-	-	12.00
1964	147.00	-	110.00	-	28.00
1965	335.00	-	770.00	-	27.00
1966	329.00	137.00	5 246.00	-	211.00
1967	244.70	335.00	265.80	-	72.70
1968	380.80	182.70	1 571.40	89.00	170.80
1969	136.00	227.50	1 040.30	490.20	289.30
1970	888.30	5.50	1 076.50	1 373.20	463.90
1971	45.90	24.30	1 125.80	2 283.50	254.00
1972	80.20	137.40	1 480.40	1 804.50	219.40
1973	52.60	64.20	1 587.10	1 296.60	250.70
1974	8.60	53.20	1 446.20	1 605.60	187.70
1975	9.50	23.00	764.70	745.70	59.70
1976	10.00	43.20	1 313.60	505.50	115.70

Source: The Indonesian Directorate General of Fisheries.

By far, Ujungpandang has been the major market and shipping harbor for seaweeds harvested mainly from the south coast of Celebes and the adjacent islands. In recent years, harvests from Nusa Tenggara (Sumbawa, Flores, Sumba) and the Moluccas region have also been transported to Ujungpandang. The seaweed production from the Moluccas now is rather substantial (over 2 000 mt in 1971) to make Ambon another center of seaweed trade.

Between 1920 and 1930, Indonesian seaweeds exported almost exclusively to China consisted chiefly of *E. spinosum*. The export statistics for 1930-1940 also indicates that 20-30% of the exported seaweeds landed in Japan and consisted mainly of *G. blodgettii*, *G. lichenoides*, *G. amansii* and *G. rigidum*. Singapore and Hong Kong are important transitory ports of

seaweed exports destined for European, American, or even Japanese markets. However, in the last few years, direct exportation has increased steadily.

SEAWEED FARMING

One of the problems of the seaweed export industry is the lack of a constant supply of seaweeds of good quality. Shipments containing some low-grade seaweeds are unacceptable in the international market and are usually sold at low prices. Steps are being taken to remedy this problem.

Some commercial seaweed buyers are sponsoring programs on the cultivation of *E. spinosum* in tropical Southeast Asian countries such as Singapore, Philippines, and Indonesia. Successful field cultivation will ensure yields of predictable quantity and quality and possibly also reduce transport costs. So far, the results have been very encouraging and the Philippine experience has become a model of such efforts (Doty 1973).

Indonesian seaweed culture in the laboratory started as early as 1966 (Soerjodinoto 1968, Soegiarto 1968, Ismail 1971) but the study progressed only slowly due, in part, to political and economic instability in the country during that time. In 1972 the study was resumed with strong government support, especially by the Indonesian National Institute of Oceanology (NIO) and the Marine Fisheries Research Institute (MFRI). In the last few years, a number of seaweed farms have been set up by NIO at Pari Islands, Jakarta Bay (Soegiarto et al. 1975) and the Tanjung Benoa, Bali (Sulistijo and Atmadja 1980); by MFRI and Copenhagen Pectin Company in Samarṅginga Islands, Central Celebes (Mubarak 1975); by fishermen in North Tanimbar Island and in Maumere (Mubarak 1974); by MFRI and Marine Colloids in Riau (Mubarak 1976); by Directorate General Cooperative and USAID in Aru Islands, Moluccas (Mubarak 1978); by fishermen in cooperation with the Moluccas Government and NIO Ambon Station in Geser, Ceram Island, Moluccas; and by fishermen with the assistance of Copenhagen Pectin Company in Terora-Tanjung Benoa, Bali (Sulistijo and Atmadja 1980).

The *E. spinosum* farm in Terora-Tanjung Benoa, Bali, so far has produced the best results, with a monthly production of 15-20 mt dry weight in 1979. However, due to the decreased prices, the monthly production declined to around 10 mt after one year. Furthermore, experimental results have shown that the growth rate on floating rafts is slightly better than that of the bottom method. For example, in the farms in Tanjung Benoa, Bali, the average growth rate was 4-5%/day on the floating rafts and less than 3%/day in the bottom method.

CONCLUSIONS

The economic value of seaweeds in Indonesia is not yet fully appreciated. The full potential of the country's seaweed resources remains to be tapped.

More extensive surveys and research are urgently needed to determine the potential production and the factors which govern the productivity of the economically important species.

Only a few species have so far been investigated for their nutritional value and chemical composition which considerably vary from one species to another and with geographical distribution and seasonal changes.

The expansion and upgrading of agar factories in Indonesia will depend on government protection against imported products, capital investments, improved management, continuous supplies of good quality raw materials, and the application of more modern technology.

Seaweed exports can be increased through attractive export regulations and bonuses in combination with strict quality control.

Seaweed farming should be encouraged to increase production, control the quantity and the quality of seaweeds, and reduce interinsular transport costs and risks. Technically, seaweed farming is easy and does not require much capital investment. It will also provide new job opportunities for fishermen.

Appropriate marketing and pricing systems should be developed in order to protect the seaweed farmers from unscrupulous dealers or buyers.

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PRESENT STATUS OF SEAWEED CULTURE IN KOREA

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ABSTRACT

Seaweeds from natural stocks as well as from aquaculture have been widely utilized in Korea for a long time. *Porphyra* was first cultivated 360 years ago. The culture of *Undaria pinnatifida* was introduced more than 10 years ago. *Laminaria* spp. were also introduced by employing an artificial culture method. *L. religiosa* was grown in natural beds along the middle part of the eastern coast after the initiation of farming of this species. At present, production of *U. pinnatifida* from farms is much greater than the natural harvest.

The most widely cultured species of *Porphyra* is *P. yezoensis*, but *P. tenera* is also farmed in some areas. Recently, some varieties that were known to grow faster and to be more resistant to diseases were introduced from Japan. After *Conchocelis* was successfully grown, the artificial seeding method became very popular.

The production of cultured *Porphyra* was 34 025 mt in 1980; *U. pinnatifida*, 153 333 mt in 1979; *Laminaria*, 5192 mt in 1979.

INTRODUCTION

Seaweeds from natural stocks and aquaculture have been widely utilized in Korea for a long time now. Chung (1814) described thirty-four species of seaweeds with particular emphasis on their use as human food.

Porphyra was first cultivated on tidal flats of Kwang-Yang Bay near the estuary of the Seom-Jin River 360 years ago. Later it was grown on shrub branches standing on tidal flats. Since then more efficient culture methods have been developed. Split-bamboo blinds replaced the shrub branches as culture substrates. Initially, one lateral margin of the split-bamboo blind was fixed to the sea bottom and the other side was left free in the water at certain level within the tidal range. In the 1930's, this method was modified by setting the split-bamboo blind substrate in a horizontal position.

Undaria pinnatifida has been harvested for a long time from natural rocks by using a method that eliminates 'pest' weeds such as *Sargassum Phyllospadix*, and articulated coralline. The cultivation of the species started in 1970 and at present the production from cultures is much higher than natural harvests.

Laminaria spp. are distributed along the eastern coast of northern Korea. In the south, where it is too warm for the natural growth of the species, *L. japonica* and *L. religiosa* have been introduced employing an artificial culture method. *L. religiosa* later started to grow on natural rocks along the middle part of the eastern coast sometime after the initial farming of this species.

As these species of sea vegetables are now grown extensively by a number of sea farmers, seedling production which is undertaken by highly skilled farmers is a major concern of the seaweed industry in South Korea.

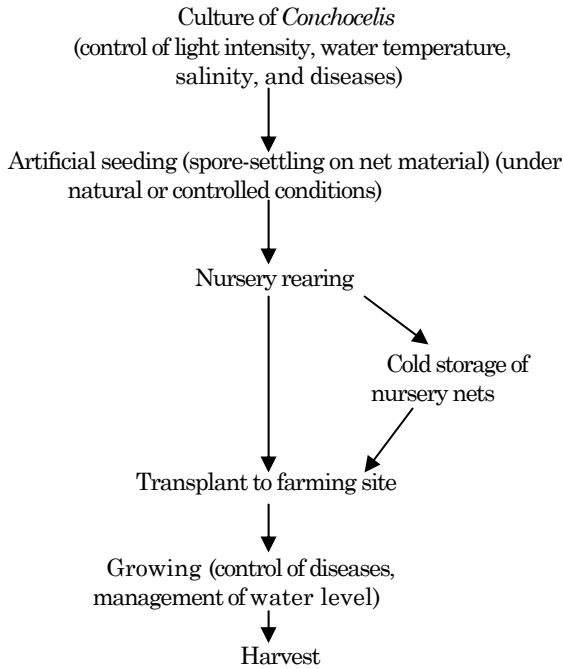
PORPHYRA CULTURE

Among *Porphyra* species, *P. yezoensis* is at present most commonly cultivated although *P. tenera*, which used to be more popular, is still grown in some farms (Kang 1970). Recently, some varieties that grow faster and are more resistant to diseases were introduced from Japan. The limited success in the cultivation of these varieties may be due to differences in environmental conditions and the lack of experience of farmers in culturing new cultivars.

About 4 to 5 years ago, *Conchocelis* was cultured by the free-growing method before the technique of substrate culture in shells. However, farmers generally do not utilize this method. They obtain their seeds from the National Fisheries Research and Development Agency of Korea or import them from Japan to get new strains.

Before the technique of artificial seeding from *Conchocelis* cultures was developed about 10 years ago, farmers obtained their seeds from natural spores settling in the field. Now, the old method of natural spore-settling is no longer practiced. Artificial seeding is usually carried out in large plastic envelopes. The cultured *Conchocelis* and nets for spore-settling are placed inside the envelope containing sea water. The envelope is then set under water in the field to simulate or approximate natural conditions. Since seeding requires exact timing of spore release, farmers usually prefer artificial seeding under natural conditions than under fully controlled conditions in tanks installed on land.

The *Porphyra* culture method is as follows:



The nets are then suspended in seawater during the nursery growing period of about 55 days. The grow-out nursery nets are almost directly transplanted to the final growing ground. Only very few farmers keep some of the nursery nets refrigerated.

The old practice of using split-bamboo blinds as culture substrate has been substituted by the net-culture method which is more convenient and efficient for spore-settling. The seeded nets (1.8 × 4m) are usually placed and maintained at a certain level within the tidal range by attaching them to supporting poles. The nets float near the water surface and are exposed to the air twice a day during the spring low-tide period. The average time of each exposure is 3 to 4 hours.

If the exposure time is short during the early stages of the culture period, *Porphyra* grows fast but is more vulnerable to diseases. On the other hand, with a long exposure period it grows slowly but is more resistant to diseases. Moreover, when warm temperatures accompany the calm weather during December, it is very difficult to grow healthy *Porphyra* by adjusting the exposure time alone. However, farmers can grow *Porphyra* fast and increase yields by reducing the exposure time in December. But the next crop usually suffers from diseases.

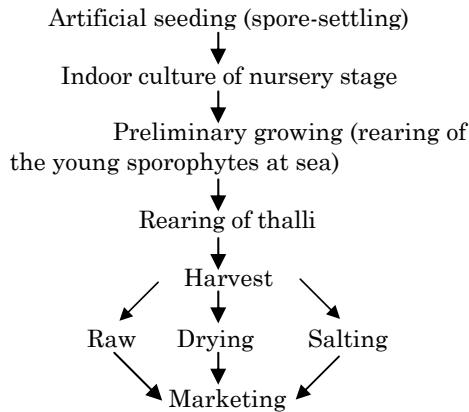
The nets in the drift system are always floating on the surface of the water and maintained in place by anchors. This culture system is beginning to be practiced toward the open sea because of the pollution of inland seawaters. Recently, production of *Porphyra* has expanded to meet increasing demands. However, there is the need to improve its quality.

UNDARIA CULTURE

Korea has two species of *Undaria*: *U. pinnatifida* and *U. peterseniana*. Because the distribution of the latter is restricted to Cheju Island and its vicinity, it is not as popular as the widely distributed *U. pinnatifida* which is preferred by the Korean people. The thalli of another species, *Costaria costata*, is also utilized as food.

The culture technique of *Undaria* was introduced more than ten years ago in the vicinity of Pusan (Saito 1964) which is located along the country's southeastern coastline. About 70% of the total production of the species in Korea today, however, is produced along the southwestern coastal areas.

The culture and production of *Undaria* involve:



Artificial seeding is usually carried out from late May to early June when the seawater temperature increases to around 17°C. When shade-dried sporophylls are immersed in seawater, the zoospores are discharged. The zoospores germinate into gametophytes which grow very fast up to the young sporophyte stage when the water temperature is below 20°C until July before the onset of high water temperature. If light intensity is reduced, gametophytes grow only into a few cells. Growth of gametophytes is slow until August since water temperature thereafter begins to increase.

The success of *Undaria* culture basically depends on the development of the young sporophytes. Factors like high water temperature and transpar-

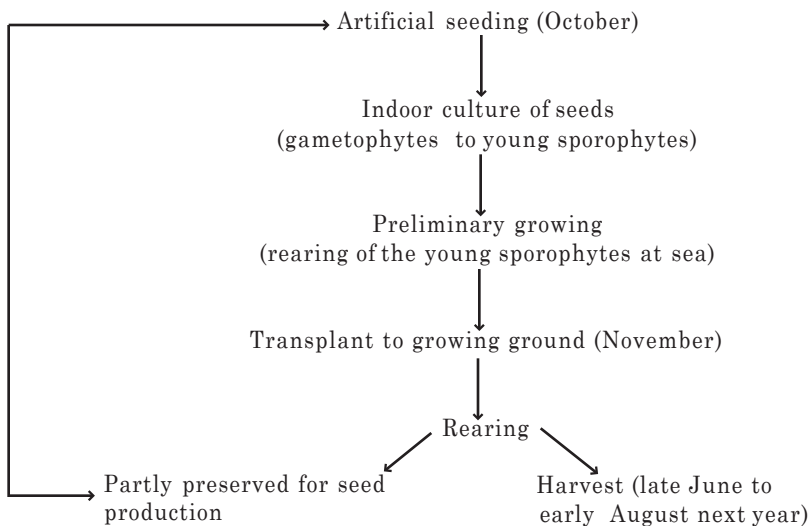
ency as well as unstable sea conditions owing to prevailing strong winds and fluctuation of water temperatures apparently influence the growth of young sporophytes. Recently, certain diseases caused by bacterial pathogens and parasites, such as *Thalestris* sp. and other copepods, have been observed in cultures.

The market price of the dried product fluctuates considerably. At the very start of the harvest season in December, it is about ten times as much as in February to April. Therefore, some farmers start to grow young sporophytes early in September, in spite of the adverse effects of warm temperatures, so as to harvest in December when prices are high. However, when fully grown sporophytes experience high water temperatures during the hot season, survival of the sporophytes is low.

LAMINARIA CULTURE

Although *Laminaria* generally thrives in cold temperate waters, it is now possible to farm it in warmer temperate waters through artificial seed production (Li 1969). In this method, spores are collected on strings from blades of mature *Laminaria* in May and then kept in the dark and at low temperature indoors during summer in Japan. They are moved to the sea in autumn and then grown to marketable size by July in the following year. When cultured from spores, the seaweed still behaves as a biennial and develops the same thickness and taste as naturally grown individuals. *Laminaria* is now cultured in South Korea in areas other than its natural habitats.

The culture of *Laminaria* in Korea consists of the following:



In Korea, seedling culture begins in October and the rearing of young sporophytes is carried out from late October to November when the water temperature is below 18°C. It is harvested by late July before the water temperature rises to 20°C. In summer, typhoons may damage seaweed farms, and the cultures may be affected by the growth of bryozoans.

PRODUCTION OF CULTURED SEAWEEDS

The data on the production of cultured *Porphyra*, *Undaria*, and *Laminaria* in South Korea in 1975-1980 are given in Table 1. Annual total harvests for each seaweed during this period varied greatly, but *Undaria* clearly constituted the bulk, followed by *Porphyra* and *Laminaria*. *Porphyra* production

Table 1. The production of cultured seaweeds in South Korea

Species	Production (metric tons)					
	1975	1976	1977	1978	1979	1980
<i>Porphyra</i>	-	29 090	11750	23 610	29 240	34 025
<i>Undaria</i>	132 328	161 770	208 620	158 342	153 333	-
<i>Laminaria</i>	2 759	8 342	2 122	5 516	5 192	-

was a high 34 025 mt in 1980. *Undaria* and *Laminaria* accounted respectively for 153 333 and 5 192 mt in 1979; no data are available for both seaweeds for 1980.

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UTILIZATION OF SEAWEEDS IN THAILAND

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ABSTRACT

Different seaweeds harvested from natural stocks are utilized in Thailand as human food and animal feed and for medicinal purpose and extraction of crude agar. *Gracilaria* and *Porphyra* are the most exploited commercially. Commercial cultivation through seaweed farming is recommended.

INTRODUCTION

Thailand, which lies between Latitudes 5° to 21°N and Longitudes 97° to 160°E is one of the countries in Asia favorable for the growth of seaweeds. Being an entirely tropical monsoon country, pronounced wet and dry seasons characterize the climate of the land. The rainy season at the upper part of the country is from May to October, while in the south the rainy period is from October to February. The dry season begins in November and lasts until April. Thailand has 2 527 kilometers of coastline which abounds with seaweeds. The southern coastlines border the Gulf of Thailand on the east and Andaman Sea on the west and consist of sandy-rocky shores, coves, and mangroves areas. However, not much Phycological work has been done along the sea coast.

SEAWEED UTILIZATION

The Thai people use seaweeds as food, as animal feed, for medicinal purposes, and for extracting agar. Seaweeds are eaten only in particular areas, especially along the coast of the Gulf of Thailand and Andaman Sea. The majority of edible seaweeds belong to the genera *Gracilaria*, *Porphyra*, *Caulerpa*, *Sargassum*, *Hypnea*, *Laurencia*, *Acanthophora*, *Padina*, *Dictyota*, *Hydroclathrus*, and *Chaetomorpha*. These seaweeds are consumed fresh or blanched as salad vegetables, mixed with some ingredients, or used in soup preparations (Lewmanomont 1978).

Gracilaria is the only genus used for agar extraction. To extract agar, local people boil in water the bleached, dried *Gracilaria*, filter the mixture through muslin, and let the filtrate set into a gel. Agar can be made into different desserts (Boon-nag 1935).

Seaweeds used for medicinal purpose are *Sargassum* and *Laurencia*. Both are used in the treatment of goiter. Dried *Sargassum* is also boiled and taken as tea to lower body temperature when one has fever.

For animal feed, only the green seaweed *Ulva reticulata* is used in the diet of pigs.

Among the useful seaweeds, *Gracilaria* and *Porphyra* are more popular than the other genera. Both are exploited commercially, but are harvested only from natural stocks.

Gracilaria

This genus occurs in many areas in Thailand. More than ten species had been reported (Lewmanomont 1978). The most common species, *G. verrucosa*, is widely distributed in the Gulf of Thailand and Andaman Sea. The other common species are *G. blodgettii* and *G. crassa*.

Based on the report of the Department of Customs (1956-1980), Thailand exported *Gracilaria* to many countries for agar extraction in 1956 to 1961 and again from 1975 to 1980. In 1980, Thailand exported more than 200 tons of dried *Gracilaria* to Japan, Federal Republic of Germany, Italy, and Hong Kong. Only a small volume was utilized locally as food and for extracting agar. During the same period, Thailand imported agar from Japan, Hong Kong, Korea, Argentina, United States, United Kingdom, and Federal Republic of Germany. It seems ridiculous to export seaweed raw material abroad and then import the final product, agar. In 1966, Thailand imported only 66 tons of agar. Since then, imports have increased dramatically every year. In 1979, 225 tons of agar worth 67 million baht was imported. Therefore, if the cultivation of *Gracilaria* in Thailand becomes successful, it will increase the income of the Thai people living along coastlines and also minimize agar imports once an agar-extracting factory in Thailand is established.

Porphyra

This genus is an expensive red seaweed used as food in Thailand. The common species is *P. vietnamensis*. This species occurs only in the south at

Songkhla, Pattani, and Narathiwat during November to February when the salinity and temperature of seawater are low. It grows on exposed rocks constantly splashed by waves. The local people collect *Porphyra* by hand and sell it fresh in the market or dry it into sheets. The annual production is variable and depends on environmental conditions. It is only at Songkhla where the alga is commercially exploited. The annual yield is about 500 kg fresh weight (Lewmanomont and Ogawa 1979; Prommanond and Sahawatcharin 1968; Thiemmedh 1960). Since *P. vietnamensis* is a tropical species that can tolerate high temperatures, its commercial cultivation in Thailand is possible.

PROSPECTS OF SEAWEED FARMING

Seaweed farming can provide a steady supply of raw material to a seaweed industry. The potential of seaweed farming in Thailand is rather high since favorable environmental conditions therein such as high light intensity and temperature throughout the year support good seaweed growth. The productivity of seaweed farming is higher in warmer areas than in cold regions. This may be due to faster seaweed growth rates and longer growing seasons in warm areas. Moreover, seaweeds of commercial importance occur in Thailand which are easier to culture than introduced species.

Gracilaria and *Porphyra* offer the best prospect for seaweed farming in Thailand. A well-planned project is seriously needed. Cooperation with other countries and aid from foreign specialists are also required.

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LIFE HISTORY OF *ACROTHRIX PACIFICA* AND *SPHAEROTRICHIA DIVARICATA* IN LABORATORY CULTURES

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ABSTRACT

The life histories of *Acrothrix pacifica* Okamura & Yamada (Acrothricaceae) and *Sphaerotrichia divaricata* (Ag.) Kylin (Chordariaceae) in the Chordariales, Phaeophyceae were studied in the laboratory. Both species showed an alternation of macroscopic sporophyte (2n) and microscopic gametophyte (n).

In *A. pacifica*, unfused gametes developed into haploid sporophytes under cooler conditions or into gametophytes under warmer conditions. In *Sphaerotrichia pacifica*, unfused gametes developed into gametophytes under warmer conditions.

INTRODUCTION

The Chordariales in Japan contains four families and seventeen genera: Leathesiaceae (*Petrospongium*, *Cylindrocarpus*, *Leathesia*), Chordariaceae (*Chordaria*, *Cladosiphon*, *Eudesme*, *Haplogloia*, *Heteroscaudersella*, *Myriogloia*, *Analipus*, *Papenfusiella*, *Pseudochorda*, *Sauvageaugloia*, *Saundersella*, *Sphaerotrichia*, *Tinocladia*), Spermatochnaceae (*Nemacystis*), and Acrothricaceae (*Acrothrix*). Most of the genera and species in this order are distributed in the northern part of Japan, except for *Cladosiphon okamuranus*, a locally edible species which grows in the subtropical waters of Okinawa and Amami Islands. Other edible species such as *Nemacystis decipiens*, *Tinocladia crassa*, *Sphaerotrichia divaricata*, and *Acrothrix pacifica* are distributed along the Pacific Ocean and Japan Sea coasts in Honshu and western and northern Kyushu. These five species have been used as "sea vegetables" in Japan.

The life histories of *N. decipiens* and *T. crassa* have been studied by Yotsui (1975, 1980), Yotsui and Migita (1974) and Yotsui (1978, 1979a, b), respectively. Shinmura (1974, 1975, 1976, 1977a, b) and Shinmura and Yamanaka (1974a, b) studied the life history and ecology of *C. okamuranus*. Based on these studies, *N. decipiens* and *C. okamuranus* are now artificially cultivated in the sea to increase production.

Since an understanding of the life history is fundamental to the cultivation of algal species in the sea, a study was conducted on the life history of *A. pacifica* and *S. divaricata* in the laboratory. In laboratory cultures, the life cycles of these two species were previously found to be heteromorphic, with an alternating macroscopic sporophyte and microscopic gametophyte (Ajisaka 1979; Ajisaka and Umezaki 1978). Furthermore, under given conditions, the sporophytic and gametophytic stages were alternately repeated without producing any further generations.

MATERIALS AND METHODS

The sporophytes of *A. pacifica* and *S. divaricata* were collected at Takahama in Wakasa Bay, which is in the middle part of Honshu facing Japan Sea. The fertile sporophytes bearing unilocular sporangia were collected during the early summer months of June and July. The isolation of zoospores discharged from the sporophyte was done by the micropipette method. The culture medium used in this study was Provasoli's ES medium. Cultures were illuminated with cool white fluorescent lamps (ca. 1500-3000 lux) and were incubated under the following temperature-photoperiod regimes: 20°C: 18-6 hr (set 1); 20°C: 10-14 hr (set 2); 15°C: 14-10 hr (set 3); 15°C: 10-14 hr (set 4); 10°C: 14-10hr(set5). For the *A. pacifica* cultures, there were two additional regimes: 10°C: 10-14 hr (set 6); 5°C: 10-14 hr (set 7).

RESULTS AND DISCUSSION

Acrothrix pacifica

The unilocular sporangia of *A. pacifica* are elongated obovoid 44-66 x 25-41 (32 x 55 average) μ m in size. Zoospores discharged from the unilocular sporangium are pear-shaped, with a single chromatophore and an eyespot, and laterally biflagellated. Soon after settling on the substratum (glass slide), zoospores became spherical with a diameter of 4.4-7.6 (5.6 average) μ m.

The settled spores germinated and developed into creeping uniseriate filaments on which hyaline hairs were produced. Under warmer conditions

(sets 1 and 2, 20°C), the creeping filaments developed into prostrate and upright systems forming dense tufts. Under cooler conditions (sets 5 and 6, 10°C), the creeping filaments developed into comparatively smaller tufts and produced many larger erect filaments from the center. Each erect filament profusely branched off on the opposite side or on all sides. At 10°C, most of the cells of the branches and branchlets of erect filaments were transformed into uni- or biseriate plurilocular gametangia. The gametophytes mature within 2-3 months under set 5 (10°C: 14-10 hr) and within 3 months under set 6 (10°C: 10-14 hr).

The gametes from the gametangium are pear-shaped, measuring 5.7-10.2 x 3.0-5.3 μm . Under cooler conditions (sets 4-7, 10-15°C), gametic conjugations (usually isogamous, rarely anisogamous) were observed. The fused gametes settled on the substratum (glass slide) and soon became spherical. The settled zygote germinated to produce filament that later developed into a monosiphonous central axis. Each cell of the central axis divided giving rise to a primary assimilating filament. Then some basal cells of the primary assimilating filaments divided to produce a medullary layer which grew trichothallically into a cylindrical plant. Under cooler conditions (5-10°C), the cylindrical plant branched off laterally and after 3 months, it matured producing many unilocular sporangia which released zoospores. The branched fertile plants are similar in habit to sporophytes from the sea.

The gametophytes derived from zoospores of sporophytes carry 8-14 chromosomes while the sporophytes from zygotes of cultured gametophytes have 14-19 chromosomes (Table 1). This indicates that the sporophytes are diploid and the gametophytes are haploid. Unfused gametes germinated into sporophytes under cooler conditions (10°C). The sporophytes have 8-14 (of which 50% has 9) chromosomes and, therefore, seemed to be haploid. The haploid sporophytes bore unilocular sporangia and released zoospores. The zoospores directly germinated and gave rise to haploid gametophytes. On the other hand, under warmer conditions (sets 1 and 2, 20°C), most of the unfused gametes germinated into haploid gametophytes, repeating the same generation. Under moderate conditions (set 3 and 4, 15°C), the unfused gametes germinated into sporophytes (more in number than gametophytes). When one-celled germlings of unfused gametes were cultured under cooler conditions (10-15°C), they germinated into sporophytes which, however, grew smaller than normal diploid sporophytes and died within a month. On the other hand, when the unfused gametes under warmer conditions (10-15°C) were transferred into cooler conditions (5-10°C), they again germinated into gametophytes.

This study indicates that the life history of *Acrothrix pacifica* is morphologically an alternation of macroscopic sporophytes with microscopic gametophytes or, karyologically, a diploid sporophyte alternates with a haploid gametophyte. Furthermore, unfused gametes developed into haploid sporophytes under cooler conditions and into gametophytes under warmer condi-

Table 1. Chromosome number of *Acrothrix pacifica*

Karyology:	diploid	haploid	haploid
Plant:	sporophyte from zygote in culture	gametophyte from zoospore in culture	sporophyte from unfused gamete in culture
Chromosome number:	14-19	8-14	8-14 (50% is 9)

tions. This culture study also suggests that the sporophytes prefer the cooler waters of winter and spring. On the other hand, gametophytes, which have not yet been found in the sea, prefer the warmer waters of summer and autumn (Fig. 1).

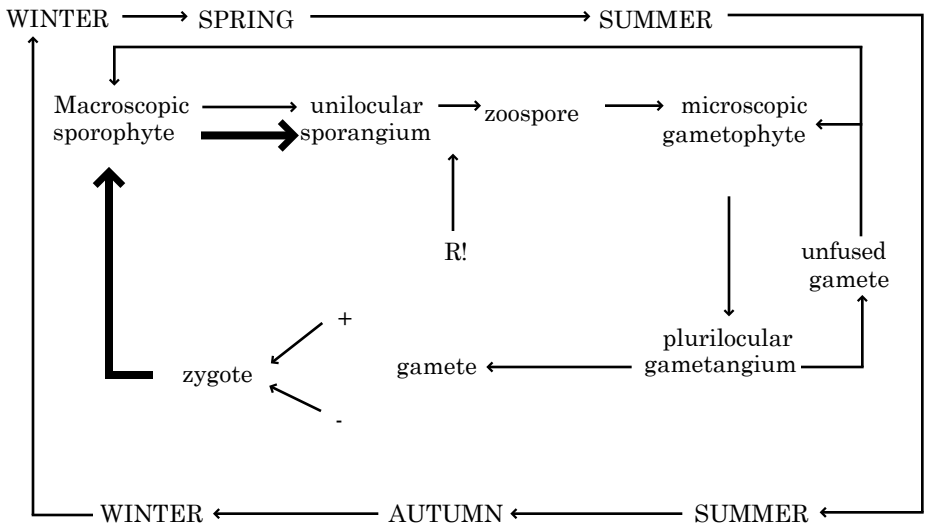


Fig. 1. Life history of *Acrothrix pacifica* (—haploid, **—** diploid, R! meiosis).

Sphaerotrichia divaricate

S. divaricate grows epiphytic on *Sargassum confusum* and *S. piluliferum* or on rocks 1-2 m below low tide mark. The fertile sporophytes have unilocular sporangia. The sporangia are elongated obovoid measuring 61-75 x 26-41 μ m. Zoospores discharged from the sporangium are pear-shaped with a single chromatophore and an eyespot 4.7-6.6 x 2.8-3.8 μ m in size, and laterally biflagellated. Soon after settling on the substratum (glass slide)

they became spherical with a diameter of 2.8-4.8 μm . Later, they germinated and developed into creeping filaments. They branched laterally, forming dense tufts composed of prostrate and upright systems. Under warmer and long-day conditions (set 1, 12°C: 16-18 hr), the upper parts of some erect filaments were transformed into uniseriate plurilocular gametangia within 13 days. However, under set 2 conditions of 20°C: 10-14 hr, the tufts were larger (over 1 cm dia.) and never bore plurilocular gametangia even after 2 months. Under cooler (10°C) and long-day (14-10 hr) conditions, 8-day old filaments which are usually provided with hairs, developed into simple tufts composed of small prostrate and sparsely branched erect systems. Within 20 days they matured and formed plurilocular gametangia. Under moderate conditions (set 3, 15°C: 14-10hr; set 4, 15°C: 10-14 hr) two types of tufts were formed: dense tufts at 20°C (sets 1 and 2), and simple ones at 10°C (set 5). These two types were formed at nearly equal rates and matured and started liberating gametes within 18 days.

The gametes discharged from the gametangium were morphologically similar to zoospores, laterally biflagellated, and measured 4.7-6.2 x 3.3-5.2 μm in size. The discharge of gametes was induced when cultures were transferred from dark to light. The conjugation between gametes was isogamous or rarely anisogamous. While the rate of the gametic conjugation was 70-80% under cooler conditions (set 5, 10°C), the process scarcely occurred under warmer conditions (sets 1 and 2, 20°C) and most of the gametes germinated without fusion and each developed into a gametophyte, repeating the same generation.

The zygotes became spherical and soon germinated to develop into cylindrical plants consisting of assimilating filaments, cortical and medullary layers. Under cooler conditions (set 5, 10°C: 14-10 hr), the erect cylindrical plants branched off and grew larger, with a habit similar to that of sporophytes in the sea.

Unfused gametes germinated and developed into gametophytes. The gametophytes grew faster and became fertile earlier under warmer and long-day conditions (set 1, 20°C: 16-18 hr) than under cooler and short-day conditions (set 6, 10°C:10-14 hr; set 7, 5°C: 10-14 hr.).

The chromosome number of sporophytes from the sea was 23-30 and that of gametophytes derived from zoospores was 7-17. Eighty percent of the gametophytes, however, had a chromosome number of 9-12 (Table 2). Although the sporophytes derived from conjugated gametes in laboratory culture had 15-27 chromosomes, 90% of them had 18-24.

This culture study has demonstrated that cooler conditions favor sporophyte growth while warmer temperatures induce sporophytes to produce unilocular sporangia earlier. Moreover, the study has confirmed that the life history of *S. divaricata* is heteromorphic, an alternation of diploid macro-

Table 2. Chromosome number of *Sphaerotrichia divaricata*

Karyology:	diploid	haploid	diploid
Plant:	sporophyte from sea	gametophyte from zoospore in culture	sporophyte from conjugated gametes in culture
Chromosome number:	23-30	7-17 (80% is 9-12)	15-27 (90% is 18-24)

scopic sporophytes and haploid microscopic gametophytes. Meiosis may occur during the formation of zoospores in the unilocular sporangium on the diploid sporophyte (Fig. 2).

The results of culture experiments also suggest that although gametophytes have not yet been found in the sea, both gametophytes and sporophytes are present. In summer, gametophytes grow well and mature faster. Most of the gametes germinated without conjugation, repeating the same gametophytic generation. While the rate of sexual conjugation increases from autumn to winter when seawater temperature drops, the sporophytes derived from zygotes develop well into branched macroscopic fronds during

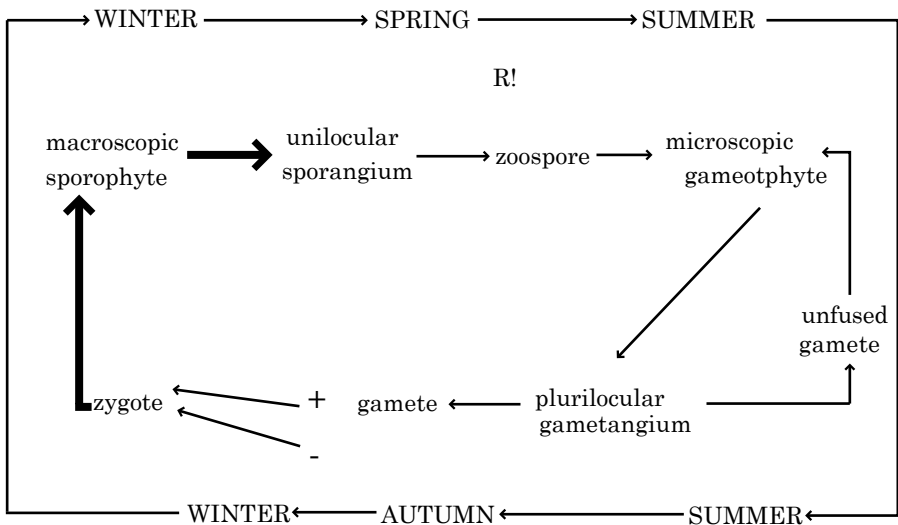


Fig. 2. Life history of *Sphaerotrichia divaricata* (— haploid, — diploid, R! meiosis).

winter and spring. The macroscopic sporophytes bear unilocular sporangia in early summer when seawater temperature rises.

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**ANTITHAMNION SPARSUM,
ITS LIFE HISTORY AND HYBRIDIZATION WITH
A. DEFECTUM IN CULTURE**

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ABSTRACT

Antithamnion sparsum Tokida isolated from the southern and western coasts of Korea showed a basically *Polysiphonia*-type life history. However, it sometimes exhibited a monoecious reproduction and the carpospores released from the cystocarp by self-fertilization unexpectedly developed into plants bearing spermatangia alone. These male plants were not functional up to 60 days in culture.

The results of intraspecific crosses between populations of *A. sparsum* were successful and the hybrid carpospores gave rise to normal tetrasporophytes. On the other hand, the interspecific crosses between *A. sparsum* and *A. defectum* were only partly successful, as evidenced by gonimoblast development and the release of carpospores in case of *A. sparsum* (male) × *A. defectum* (female), but not in *A. sparsum* (female) × *A. defectum* (male). These results seem to suggest that both species are still undergoing speciation.

INTRODUCTION

Several species of *Antithamnion* have been cultured in the laboratory. These are considered to have a regular life history characterized by a tetrasporophyte, dioecious gametophyte, and carposporophyte (Drew 1955, Sundene 1959, Lee and West 1980). Some of them, however, show irregular reproductive cycles in addition to a typical *Polysiphonia*-type of life history (Sundene 1964, West and Norris 1966, Rueness and Rueness 1973). Still,

others in culture repeat the tetrasporic generation (Sundene 1962) or exhibit vegetative growth alone (Whittick and Hooper 1976).

The life history of *A. sparsum* has not been confirmed in laboratory culture. The species is expected to show a typical *Polysiphonia*-type although cystocarpic plants have not been reported in the field (Tokida 1932, 1954, Kang 1966, Noda 1970, Lee and Kim 1977). The plants are distributed from Saghalien to Korea (Tokida 1932, Kang 1966) and are closely related to *A. defectum* occurring in the Pacific North America (Tokida 1932). The two species have been distinguished by their cell dimensions, position of tetrasporangia, and gross morphology (Tokida 1932) which, however, can be modified by environmental factors (Sundene 1962). Thus, Wollaston (1971) suggested that *A. sparsum* may be con specific with *A. defectum*, and Yoshida (1981) recently proposed the former as a synonym of the latter.

In this paper the life history and reproduction of *A. sparsum* from Korea were investigated in laboratory culture, and the assessment of the species was considered by interspecific cross with *A. defectum* from the Pacific North America.

MATERIALS AND METHODS

Two isolates of *A. sparsum* were used in the study. One (#138 was obtained from the southern coast of Korea at the intertidal zone of Jamdo, Jinhae Bay (35°03'N, 128°40'E) on November 17, 1979, and the other (#238) from the western coast of Korea at Gopado, Garolim Bay (36°24'N, 126°21'E) on May 15, 1980. They were placed in cooler and transferred to the laboratory for culture. On the other hand, for interspecific cross experiments, culture strain of *A. defectum* (JAW #240, 241: Lee and West 1980) from California coast was obtained through the courtesy of Dr. J.A. West, University of California, Berkeley on October 8, 1980.

Unialgal cultures were set up using the methods of Lee and West (1980). Preculture of all isolates was maintained in 1/2 PES medium under cool white fluorescent light at an intensity below 300 lux. After 3-7 days, they were transferred to the incubation condition in full strength PES media, under 16-19°C, 800-1300 lux, 16:8 LD, using 7 x 7 cm glasswares. In order to eliminate diatoms, GeO₂ solution was added to the culture medium for a while (West 1970). The medium was usually changed every fortnight.

Tetraspores were obtained from fully mature tetrasporangia. After 24 h, the sporelings were transferred to a culture dish. Carpospores were cultured in the same manner as the tetraspores. To observe fertilization, the plants bearing cystocarps were isolated individually and cultured for a while to make sure that unfertilized young branches were newly grown. In

addition, a few excised apices of a female plant were kept singly in a glass container for observation of possible parthenogenesis.

RESULTS

The vegetative development of all isolates of *A. sparsum* was identical in the laboratory. There was also no detectable difference in the vegetative morphology of the tetrasporophyte from plants described from the field (Tokida 1932, 1954).

Vegetative Morphology

Erect thallus with prostrate base is 3-5 cm high and is attached to the glassware by means of rhizoidal filaments arising from the spherical basal cells of the determinate branchlets. The rhizoidal filaments with blunt tips are 4-8-celled. These also arise from the upper portion of the thallus. The cells of the main axis are 59 μ m broad and 348 μ m long, about six times as long as broad at maximum compared to 2-5.5 times in the field (Tokida 1932). Determinate branchlets on the main axis are opposite, usually 12-16-celled and semi-pinnately pectinate on the upper side. Indeterminate branches arise from every 3-7 segments of main branch and basically produce no branchlets at the opposite side.

Adventitious indeterminate branches sometimes arise from the basal cell of determinate branchlets. Hairs, which have not been recorded in the field, occur frequently on the terminal cell of determinate branchlets in the apical portion of the thallus associated with sexual reproductive structures. Gland cells are usually located on 2-3 cells of a pinnule and on the average measure 24 μ m long and 19 μ m broad.

Reproduction in Culture

The germination pattern of both tetraspores and carpospores isolated from the field for laboratory culture is basically identical. After attachment, the spores synchronously develop two opposite primordia. One later forms a rhizoid and the other develops into an apical cell from which the erect frond appears (Figs. 1-5). However, there is no definite sequence in the appearance of the rhizoid or the frond. In some sporelings, the rhizoidal cell, and in others, the frond, develops much later. Determinate branchlets at first arise alternately (Figs. 6, 8), or sometimes secondly (Fig. 7), with 16-18 days after germination. Later, after full growth, they are situated opposite one another.

The tetraspores grow into gametophytes within 20-30 days after germination. Spermatangial ramuli are observed early, and carposporangial plants appear about 10 days later. Gametophytes are basically dioecious in culture. Spermatangia develop in all parts of the pinnules in determinate branchlets. Each cell of a spermatangial ramulus cuts off a few spermatangial parent cells, which divide once or a few times, forming 2 to 4 spermatangia (Fig. 9).

Carpogonial branches are common in the upper to apical portion of the thallus, occurring singly or very rarely in pairs successively on the basal cell of determinate branchlets along the main axis and laterals. The small basal cell bearing the carpogonial branch grows larger than others and becomes the supporting cell. A mature carpogonial branch develops a long trichogyne (Figs. 11, 17).

It is known that a single carpogonial branch on each fertile apex usually matures into the cystocarp while the rest of the carpogonial branches degenerate (Wollaston 1968). However, in our culture, two to three carpogonial branches were not fertilized and grew into mature cystocarps at the same time. Such an occurrence has not been observed previously among the species of *Antithamnion*.

The development of the carposporophyte is basically similar to that preciously described by Wollaston (1968) and Lee and West (1980). The enlarged supporting cell, after fertilization, cuts off a characteristic dome-shaped auxiliary cell and becomes acetabuliform. The carpogonium, cutting off the trichogyne and leaving a cap cell at the top, produces a connecting cell that fuses with the auxiliary cell (Fig. 12) and through which the presumed diploid nucleus is moved into the auxiliary cell. After fusion, the auxiliary cell divides transversely to form the lower foot cell and upper central cell, which gives rise to the gonimoblast initials (Fig. 15). The gonimoblast cell is produced terminally on the auxiliary cell and at this stage the axial, supporting, and the foot cells are fused (Fig. 14). No special involucre is formed but the pinnae of axial cells below the cystocarp grow upwards, partially surrounding the mature cystocarp. The carpospores are released in thirty days after the formation of the auxiliary cell.

Carpospores grow to tetrasporophytes that produce tetrasporangia in thirty days after germination. Mature tetrasporangia are ovoid to ellipsoidal and measure 41 x 59 μ m on the average. Cruciate tetrasporangia are pedicellate in one or two cells or sessile on the upper part of the pinnae of determinate branchlets (Fig. 10) in contrast to the description by Tokida (1954) that they are pedicellate or sessile. Tetrasporangia release tetraspores in two weeks.

Thus, *A. sparsum* in culture completes its life cycle in four months.

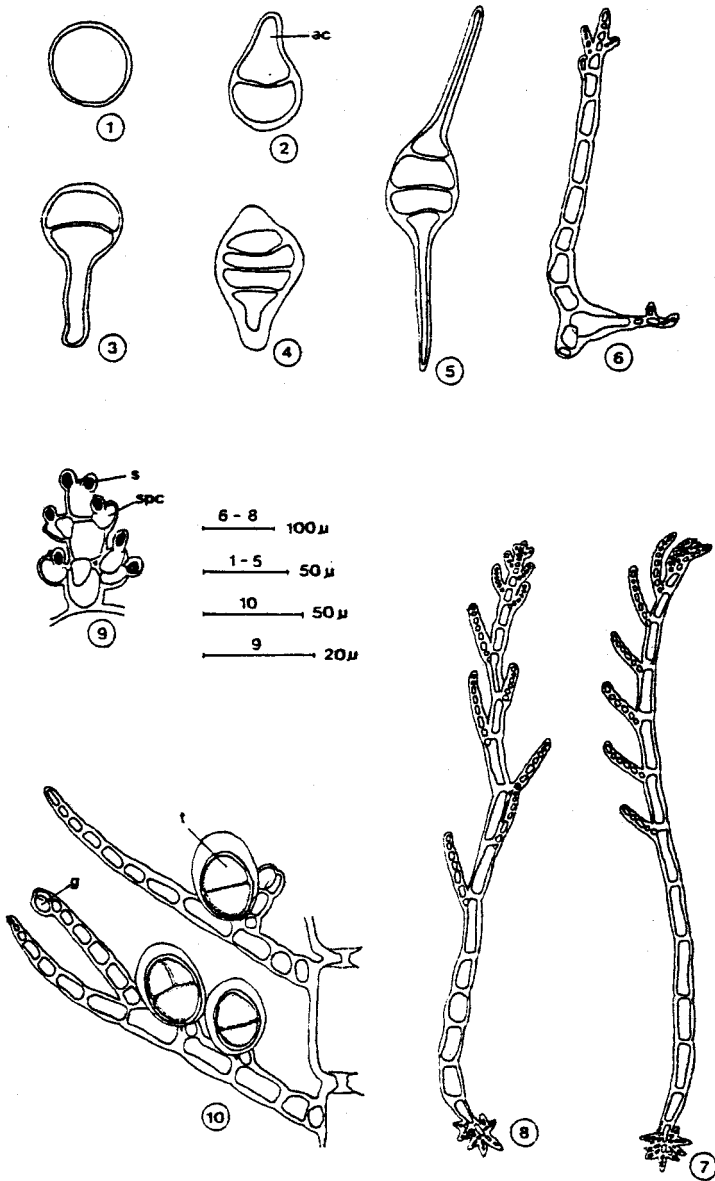


Fig. 1-10. Development of vegetative thallus, tetrasporangia and spermatangia of *Antithamnion sparsum* in culture. Fig.1. Released tetraspore. Figs. 2-3. One-day tetrasporelings. Figs. 4-5. 4-celled stage with bipolar apices. Figs. 6-8. Branching types of young plant. Fig. 9. Development of spermatangia. Fig. 10. Development of tetrasporangia (ac: apical cell, g: gland cell, r: rhizoidal cell, s: spermatangial parent cell, t: tetrasporangium).

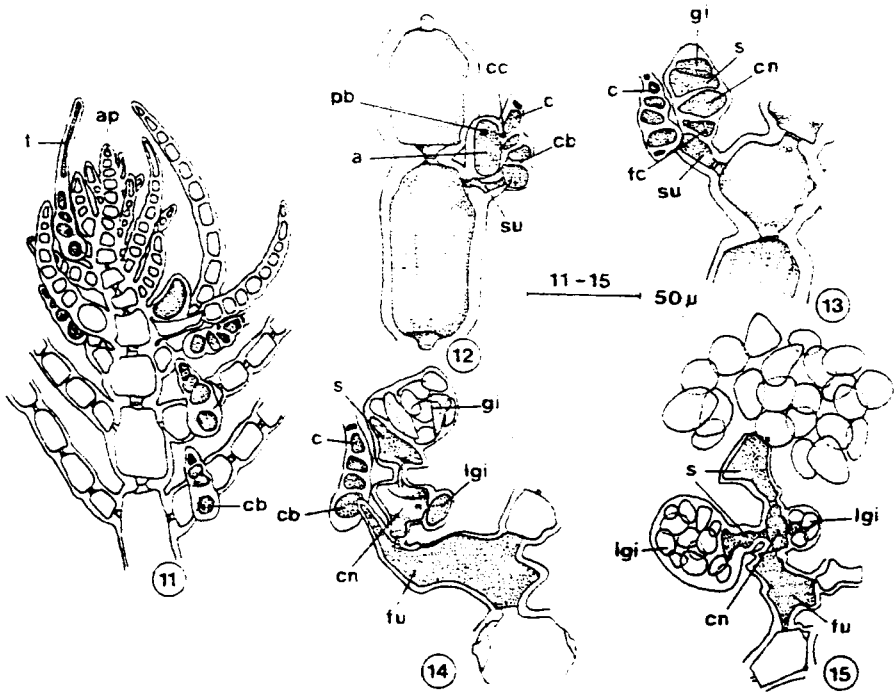


Fig. 11-15. Development of female reproductive structure of *Antithamnion spars urn Tokida* in culture. Fig. 11. Procarps in apical portion of main axis. Fig. 12. Auxiliary cell and connecting cell. Fig. 13. Development of early gonimoblast cells. Fig. 14. A young cystocarp with secondary gonimoblast initial. Fig. 15. A mature cystocarp (ac: auxiliary cell, ap: branch apex, c: carpogonium, cc: connecting cell, cn: central cell, fc: foot cell, fu: fusion cell, gi: gonimoblast initial, pb: protein body, s: sterile cell, su: supporting cell, t: trichogyne).

A typical *Polysiphonia*-type life history is repeated three times during the culture period.

Unusual Life Histories

On the other hand, several monoecious gametophytes (#138-522) derived from male gametophytes were observed in Jamdo isolates during culture. Each monoecious gametophyte, isolated individually, developed cystocarps, indicating self-fertilization. However, all the released carpospores germinated and unexpectedly grew into plants bearing spermatangia quite similar to a common male gametophyte. The fertility of the spermatangia was, however, not confirmed completely. Even though these carpospore-derived spermatangial plants were placed in culture together with normal female plants for sixty days, no cystocarps were developed.

Intra- and Interspecific Crosses

The cross between the Jamdo (# 138) and the Garolim Bay (#238) isolates of *A. sparsum* produced viable carpospores. Crosses between male *A. sparsum* and female *A. defectum* were also successful; normal cytocarps and viable carpospores were formed (Table 1). However, crosses between female *A. sparsum* and male *A. defectum* produced no mature cytocarps and viable carpospores. The gonimoblast stopped growing during early development (Figs. 16-17).

Table 1. Cross experiments among populations of *Antithamnion sparsum* from Korea and *A. defectum* from Pacific North America

Female		Male	Fertilization	Carpospore release
<i>A. sparsum</i> #138	×	<i>A. sparsum</i> #138	+	+
<i>A. defectum</i> #240	×	<i>A. defectum</i> #241*	+	+
<i>A. sparsum</i> #138	×	<i>A. sparsum</i> #238	+	+
<i>A. defectum</i> #240	×	<i>A. sparsum</i> #138	+	+
<i>A. sparsum</i> #138	×	<i>A. defectum</i> #241	+	-

* After Lee and West (1980).

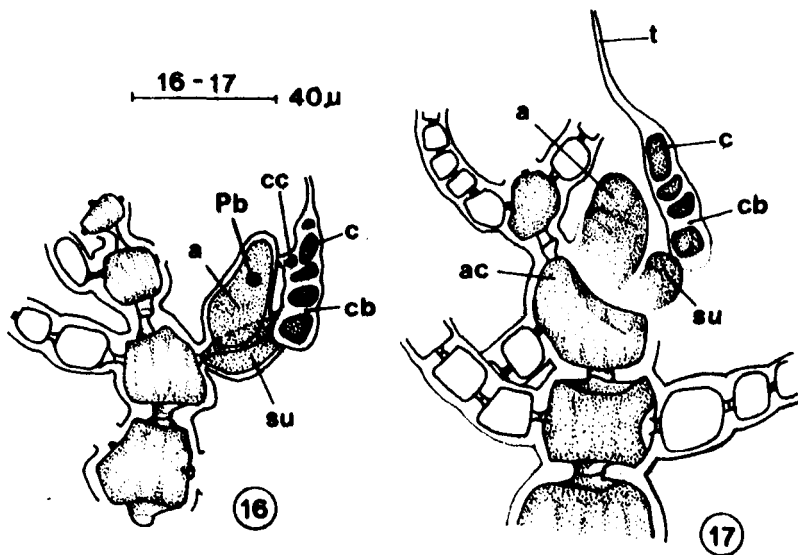


Fig. 16-17. Unsuccessful development of gonimoblast in interspecific cross of *Antithamnion sparsum* (female) and *A. defectum* (male) in culture. Fig. 16. Development of connecting cell after fertilization. Fig. 17. Development of early gonimoblast cell (a: auxiliary cell, ac: axial cell, c: carpegonium, cb: carpegonial branch, cc: connecting cell, pb: protein body, su: supporting cell, t: trichogyne).

DISCUSSION

As summarized in Fig. 18, *A. sparsum* basically shows a typical *Polysiphonia*-type of life history (Drew 1955, Sundene 1959, Lee and West 1980). Some unusual appearances of reproductive structures were also reported among *Antithamnion* species not only in culture (Sundene 1962, 1964, West and Norris 1966, Rueness and Rueness 1973), but also in the field (L'Hardy-Halos 1968, Knaggs 1969).

Monoecious reproduction is another unusual phenomenon in the life history of *A. sparsum*. Hence, the female reproductive structures were developed on the male gametophyte, and the resulting carpospores, missing the tetrasporic phase, developed exclusively male gametophytes whose spermatia were not functional. On the contrary, no female plant developed monoecious male branches in culture.

There are two previous reports on the monoecism of *Antithamnion* in culture (Drew 1955, West and Norris 1966). Thus, monoecism may not be a rare occurrence in this genus as it is also apparently common in *Callithamnion*, a related genus. However, it is peculiar that such monoecious plants in the present experiment miss the tetrasporophyte. Whittick and West (1979) demonstrated in the life history of a monoecious species of *Callithamnion* that the carpospores from the cystocarp developed into tetrasporophytes as seen in regular dioecious plants. Polanshek and West (1977) also reported the repetition of cystocarpic generations in the life history of *Gigartina papillata*. However, the lack of tetrasporic generation would differentiate *A. sparsum* from these species.

While Sundene (1962, 1964) and West and Norris (1966) reported that apomeiotic tetraspores in the gametophyte of *Antithamnion* developed only gametophytes of the same sex as the parent, Rueness and Rueness (1973) noted that the tetraspores in the male gametophytes developed into both male and female plants. A similar phenomenon also occurs in *Dasysiphonia chejuensis* (Lee and West, unpublished data). Light conditions apparently play an important role in inducing sexual reproductive structures in *Antithamnion* (Rueness and Rueness 1973).

Van de Meer and Todd (1977) reported mixed phase reproduction in the life history of *Gracilaria* sp., and suggested that the sexuality is controlled by the genetic recombination of a pair of alleles rather than a pair of chromosomes. But this was in case of diploid tetrasporophytes. They did not explain the mixed reproduction in the gametophytes observed by West and Norris (1966) and Rueness and Rueness (1973).

The monoecism of *A. sparsum* seems to be genetically stable since the female branches that develop on the male thallus are sexually irreversible and produce cystocarps successively, as in the case of tetrasporangium

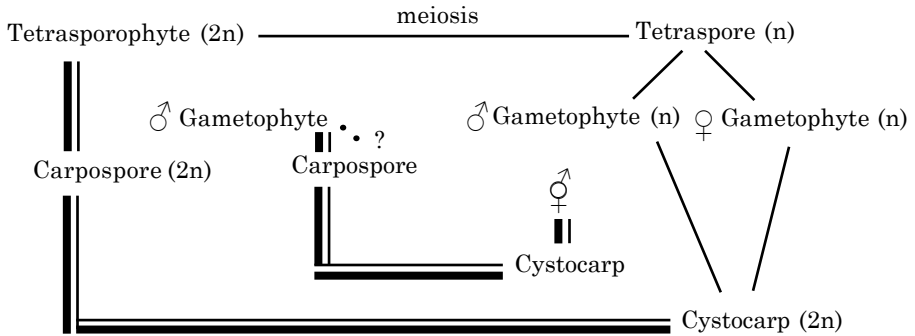


Fig. 18. Life history of *Antithamnion sparsum* in culture.

formation on cultured gametophytes of *Symphyocladia pennata* and *D. chejuensis* (Lee and West 1979). However, the presence of non-functional spermatia from such male thalli suggests that these gametophytes would be diploid rather than haploid.

In reporting *A. sparsum* as a new species, Tokida (1932) mentioned that this species showed more affinity to *A. defectum* Kylin and that both species were basically distinguished by differences in cell dimension. Wollaston (1971), therefore, doubted that both species might be conspecific and Yoshida (1981) treated the former as a synonym of the latter. In fact, such morphological characters used in separating the two species are easily subject to environmental influence (Sundene 1962, Norris and West 1967), considered vague (Wollaston 1968), and are also of little value in this study. A comparison of some significant taxonomic characters between *A. sparsum* according to Tokida and *A. defectum* Kylin is shown in Table 2.

Table 2. A comparison of some significant taxonomic characters between *Antithamnion sparsum* and *A. defectum* Kylin

Characters	<i>A. defectum</i> *	<i>A. sparsum</i> **	<i>A. sparsum</i> ***
Attachment	rhizoidal	rhizoidal	rhizoidal
Branching pattern	opposite	opposite	opposite
Cell dimension	2-3 times	2-5.5 times	5-6 times
Cell tip	tapering	blunt	blunt & tapering
Gland cell	on 2-5 cells	on 2-3 cells	on 2-3 cells
Tetrasporangia	1-2-pedicellate	1-pedicellate	1-2-pedicellate
	ovoid /80 μm	sessile /ovoid	sessile / ovoid
	long	59 × 78 μm	41 × 59 μm
Spermatangia	adaxial	adaxial	adaxial

*After Kylin (1925), Wollaston (1971).

**After Tokida (1932, 1954), Lee and Kim (1977).

***After present study.

The formation of carposporophyte and production of viable carpospores in the cross between *A. sparsum* (male) \times *A. defectum* (female) could indicate that both species are partially interfertile. However, the reciprocal cross between the female *A. sparsum* and the male *A. defectum* was not successful. They produced an auxiliary cell after fertilization, but failed to develop gonimoblast cells (Figs. 16,17) which could suggest that both species are still undergoing the speciation process.

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WATER QUALITY ASSESSMENT OF THE LANGAT RIVER, SELANGOR, MALAYSIA USING THE NATURAL ALGAL PERIPHYTON COMMUNITY AND LABORATORY BIOASSAYS OF TWO *CHLORELLA* SPECIES

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ABSTRACT

The physico-chemical conditions in ten sampling stations off the headwaters of the Langat River, Selangor, Malaysia were studied. Monitoring was done twice a month from June to December 1980. Changes in water quality were observed downstream. A total of 35 taxa of periphyton in four main divisions of algae were identified. The decrease in the number of species in downstream stations could be due to changes in the river rather than to chemical pollution. Two species of *Chlorella*, namely, *C. pyrenoidosa* and *C. vulgaris*, were grown in filtered river water obtained from the different sampling stations to assess their growth responses. Results suggest that pollution in the Langat River was caused mainly by heavy siltation rather than chemical pollutants.

INTRODUCTION

It has been documented by the Environmental Protection Society Malaysia that 42 of the country's rivers are polluted and another 23 threatened by human activities. However, only a few studies have been conducted on these rivers. Most of the water pollution programs in Malaysian rivers have been undertaken to assess the toxicity of pollutants.

This work is part of a series of surveys to study the effect of impoundment on a river ecosystem. The study assessed the quality of the waters of Langat River and determined the major cause of water pollution. Changes in the periphyton community as well as the growth of two *Chlorella* species cultured under laboratory conditions in water obtained from the river were used as indicators of water pollution. The area chosen for the study was the headwaters of the Langat River in the state of Selangor, flowing in the

geographical region of Latitudes 3°05'N to 3°13'N and Longitudes 101°47'E to 101°53'E. The river flows along the valleys of hills about 1 000 ft above sea level. The area was chosen because of: (1) the construction of the dam at the upper reaches of the river that has resulted in a change in the physical characteristics of the river; (2) an increase in human activities along the river, such as establishment of towns and a rubber-processing factory, tin mining, and paddy cultivations; (3) the utilization of water from the dam as a source of water supply going through two treatment plants, 18 miles and 30 miles away, and (4) the increase of human settlers along the river, creating domestic "tips" at various points along the river.

MATERIALS AND METHODS

Ten stations were established along the course of the river, each approximately set 4.44 km apart, except for Stations 2, 3, and 4 which were located in one area. Station 4 receives water from a mountain stream, Station 2 from the reservoir, and Station 3 from waters used to turn the turbines of a power plant operated by the National Electricity Board.

Water samples were collected at each station twice a month from June to December 1980. Other physical parameters that were measured included pH using a pH meter, air and water temperatures, current velocities, conductivity using a YSI 33-meter, and water depth and transparency. The concentrations of sodium and potassium ions in the water samples were determined using the flame photometer, nitrate-nitrogen (NO₃-N) by the Brucine method, and phosphate-phosphorus (PO₄-P) by level II of the calorimetric method. A slight modification in the phosphate determination was the use of butan-1-10 instead of hexanol. The amount of total suspended and dissolved solids was also determined at each station.

Samples of periphyton were collected from each station by scraping stones and rocks covered with algae. River water used in the culture of *Chlorella* was collected from each station in 5-liter plastic bottles. On reaching the laboratory, 25 ml of the river water was coursed through a 0.45 µm millipore filter paper into sterile flasks. One ml each of *Chlorella vulgaris* and *C. pyrenoidosa* pure cultures was washed in 5% sodium bicarbonate and then inoculated aseptically into separate flasks. There were two replicates for each water sample. The initial density of cells in the inoculum was also determined. The cultures were incubated under white light provided by two fluorescent tubes 120 cm above the flasks and a 12-hour light and 12-hour dark cycle. Growth of the cultures was quantified by counting the number of cells in each flask up to a period of seven days after inoculation, using a haemocytometer. Growth of the *Chlorella* cultures was measured using the intrinsic growth rate, r :

$$r = \frac{\ln N_t - \ln N_0}{t}$$

where: N_t = number of cells at time t ;

N_0 = number of cells at time 0;

t = maximum time when N_t occurs.

RESULTS

Analyses of the data on the physico-chemical properties of the river water showed that the pH was generally lower at the downstream stations. The highest value was recorded at Station 1 (pH = 7.6) and the lowest at Station 10 (pH = 6.2). Water temperatures were constant at the different stations except in Station 3 (21.9°C) which receives water from the power station. The water used to turn the turbines originates from streams in the mountain and is piped to the power station resulting in lower temperature. In contrast, downstream stations were characterized by higher temperature (28.5°C). Data on dissolved oxygen and carbon dioxide showed a drop in D.O. downstream after Station 8, with Station 10 recording the lowest value of 7.05 mg l⁻¹ and a corresponding increase in dissolved carbon dioxide (6.05 mg l⁻¹). The level of phosphate-phosphorus in the river water was generally low, varying from a low of 29.25 µg l⁻¹ at Station 3 to a maximum of 72 µg l⁻¹ at Station 10. Nitrate-nitrogen values fluctuated from station to station. The highest (45.17 mg l⁻¹) was recorded at Station 1; downstream stations recorded lower values.

The amount of both suspended and dissolved solids increased at the downstream stations with a corresponding decrease in the water transparency. The suspended solids increased at an average of 20 mg l⁻¹ at each station from a minimum value of 30.6 mg l⁻¹ at Station 3 to a maximum value of 193.3 mg l⁻¹ at Station 10. The amount of dissolved solids at Station 9 (91.3 mg l⁻¹) was almost double that recorded upstream at Station 3 (55 mg l⁻¹).

A qualitative survey of the periphyton flora recorded 23 species of algae belonging to four major divisions. The diatoms were the most abundant.

Bioassay studies conducted utilizing two species of *Chlorella*, *C. pyrenoidosa* and *C. vulgaris*, showed the maximum r for both species was recorded in cultures utilizing river water from downstream stations, e.g., the highest r value was recorded at Station 10 while lower values were recorded at the upstream stations.

DISCUSSION

The physical and chemical data from the ten stations indicated a change in water quality further downstream. In general, stations further downstream showed low D.O. and pH levels and high concentrations of total suspended and dissolved solids, nitrate-N₂, and phosphate-phosphorus. These observations suggest that the Langat River may already be polluted. The more acidic conditions downstream could be attributed to wastes discharged from the rubber factory as well as from domestic sources. Phosphates came mainly from detergents especially in Station 6 where the water is intensively used for daily human activities. Nitrates originated mainly from the dam as shown by the increase in its value at Station 1 as a result of increased degradation of organic matter, viz, vegetation in the newly impounded dam.

Construction work at the dam as well as road-building near Station 8 could account for the high levels of both suspended and dissolved solids that are brought downstream.

The distribution of periphyton at all 10 stations showed a general decrease; in the total number of species toward the downstream stations. However, as the environmental conditions at each station change when the river becomes deeper further downstream, the decrease in the number of species could be due to the physical change in the depth of the river rather than a result of pollution. The influence of physical changes is a common problem encountered in the use of biotic indicators of pollution in natural communities. The major parameters that determined the composition of the periphyton community in Langat River appeared to be the nature of the river beds and the velocity of the water currents.

It was hoped that the use of the laboratory culture of *Chlorella* to determine the effect of any pollutant on the test organism would minimize the problem posed by differences in environmental conditions. The higher cell numbers of *Chlorella* grown in water obtained from stations further downstream could indicate that growth of the alga was not determined by any specific chemical substance present in the water medium since there was a decrease in pH and a rise in PO₄-P and NO₃-N downstream. In preparing the medium for the culture of *Chlorella*, the river water was previously filtered. Filtration removed most of the solid particles. The change in the quality of water further downstream is apparently caused by the high levels of total suspended and dissolved solids. Pollution of Langat River, on the whole, then is due to heavy siltation as a result of dam and road constructions.

GROWTH AND DEVELOPMENT OF *TRENTEPOHLIA* *ODORATA* IN CULTURE

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ABSTRACT

Trentepohlia odorata, a filamentous green alga, grows profusely and imparts an orange color on walls of many high-rise apartment buildings in Singapore. Since bulk cultures are needed in the screening of possible chemicals to control infestation, variations in the growth and development of the alga in the laboratory under different physico-chemical conditions were investigated.

Bold's medium either as a liquid or solidified with agar was suitable substrate for the culture of the alga. An acidic growth medium (pH 4.5-6.5) favored the formation of sporangia that developed into yellowish-green colonies, whereas a basic medium (pH 7.5-9.5) produced green colonies and no sporangia. An agar concentration of 0.7% as well as the addition of thiamine to the liquid basal medium enhanced the production of sporangia developing into numerous daughter colonies after two months. High relative humidities increased colony growth and promoted the formation of yellow colonies of cylindrical cells.

On walls of buildings, the cells are elliptical to barrel-shaped, bright orange, and possess few sessile sporangia. In cultures where moisture and nutrients are not limiting, cells are narrow and elongated, with the filaments radiating from a central mass, and the sporangia are of the sessile and pedicellate types.

INTRODUCTION

Trentepohlia is a filamentous green alga easily recognized by its characteristic orange color due to an abundance of carotene in oil globules in its cells. The alga is common in the tropics, with a few species even in the temperate and sub-arctic areas. Five species have been recorded in Sin-

gapore (Johnson 1978), the more conspicuous being *T. aurea* (L.) Martius and *T. odorata* (Wigg.) Wittr. The former is commonly found on tree trunks and surfaces of rocks, forming short streaks of orange to green filaments. In the shade, the filaments may be green while under full sunlight they may turn orange. The latter forms a crustose layer on concrete surfaces and building walls, especially those of the high-rise apartment buildings, giving the surface a distinct tinge of orange (Wee and Lee 1981). *T. odorata* is of particular interest in Singapore as its proliferation on surfaces of building hastens their repainting. Hence the use of paints containing an effective anti-algal chemical may reduce infestation. Unfortunately, anti-algal paints are not as commonly available as those with anti-fungal additives. Faint manufacturers may claim that their anti-fungal products are as effective on algae as on fungi, but this has yet to be proven. Screening tests for potential algicides, undertaken locally (Ngiam et al. 1973, Ngiam and Yong 1975) as well as elsewhere (Drioko and Crylly 1974, Fitzgerald 1964) utilized blue-green algae rather than *T. odorata*, the major organism colonizing on bare walls in Singapore. Natural populations of the alga are, however, insufficient if large-scale screenings are to be undertaken. It was therefore necessary to mass-produce the alga in the laboratory for screening purposes. The following report gives an account of the influence of the pH and nutrient strength of the culture medium, vitamin supplements, and relative humidity on the growth and development of *T. odorata* under laboratory conditions. The study, hopefully, will also provide basic information necessary for controlling infestation.

MATERIALS AND METHODS

Plant Materials

Samples of *T. odorata* were scraped from walls of buildings around the former Bukit Timah Campus of the University of Singapore. A portion of the scrapings was examined under the microscope and the morphological features were noted. The rest was inoculated in Bold's basal medium contained in 125 ml Erlenmeyer flasks (Nicholas and Bold 1965). These flasks were placed under continuous lighting for three to four weeks to allow the *Trentepohlia*, together with any other algae, to proliferate. The *Trentepohlia* colonies were then isolated and reinoculated a number of times in the same medium until pure cultures were obtained. In all experiments, an inoculum of two colonies of equal diameter from the stock culture was introduced into each flask.

pH of Culture Medium

Bold's medium after autoclaving had a pH of 6.5 which served as the

control. Media of pH 4.5, 5.5, 7.5, 8.5 and 9.5 were prepared by adding dilute sodium hydroxide or hydrochloric acid to autoclaved Bold's medium.

Agar Concentration

T. odorata was also cultured in Bold's medium with various agar concentrations of 0, 0.1, 0.3, 0.5, 0.7, 1.0, 1.3, and 1.5%. To vary the agar concentration, the procedure of Hunter et al. (1966) was followed, i.e., the agar and the mineral medium of double strength were prepared separately, autoclaved, cooled to 50°C, and then mixed.

Strength of Culture Medium

Bold's basic medium of 0, 25, 50, 75, 100, and 200% strength were prepared by varying the amounts of distilled water added to the basic nutrients. The 100% or full strength medium served as the control and 0% was plain distilled water.

Vitamins, namely thiamine hydrochloride (Vitamin B₁), d-biotin (Vitamin H), and Vitamin B₁₂ were used to supplement the culture medium. In all cases, the vitamins were first dissolved in distilled water as stock solutions, autoclaved, and then added to make concentrations of 0.1, 0.2, 0.3, 0.4, 0.5, and 0.6 mg/l in the case of thiamine, and 0.2, 0.5, 1.0, 1.5, 3.0, and 6.0 mg/l for the other two vitamins.

Saturated solutions of different chemicals were used to vary relative humidities (Clayton 1967). These solutions were placed in petri dishes and a glass slide with the alga was placed above, supported by a watch glass inside. The petri dish was sealed with paraffin to maintain a constant relative humidity within the dish. The dish was placed on the laboratory bench at ambient temperature and standard lighting for 12 hours followed by 12 hours of darkness. Each was replicated thrice, using algal colonies of more or less similar size. Readings of length and breadth of cell as well as length of sporangia were made after a three-week culture period.

RESULTS

T. odorata consists of a series of subspherical to barrel-shaped to elliptical cells joined end to end in short chains. The cells are 9-17 μm long and 7-15 μm wide with smooth walls of about 1 μm thick. The prostrate filaments are more developed than the erect, and in many cases only the former are found. Sporangia are sessile, terminal or intercalary, spherical to oblong in shape, and are 10-25 μm long.

When grown in either liquid or solid culture, the filaments became cylindrical and elongated. These filaments radiated from the central core of

original subspherical to elliptical cells and branched profusely. In liquid medium, the colonies were circular and compact, showing a heterotrichous condition. The typical cells developing in culture were longer than broad, measuring 29-58 μm long and 30-5 μm wide. Under the light microscope, the cells contained less carotene than those collected from walls. The chloroplasts appeared as parietal bands although discoid forms were also observed.

In culture, sessile and pedicellate sporangia developed laterally and are generally bigger than those observed in the natural habitat. The mature sporangia possessed a characteristic beak-like protrusion at the apical end.

pH of Culture Medium

The optimum pH was 7.5 in liquid culture, based on colony size, relative abundance of new colonies formed, and dimensions of apical cells (Table 1). Generally, growth was better with increasing pH. Sessile sporangia were noted between pH 4.5 and 6.5, these being terminal at pH 4.5 and 5.5, but lateral and confined to the older cells toward the center of the colony at pH 6.5 (Table 2). At the normal pH of 6.5, the cells were cylindrical and the colony profusely branched. The few small oil globules containing carotene were clustered at the center of the cells. With decreasing pH, the cells became barrel-shaped, then elliptical, and accumulation of carotene in the cells increased. On the other hand, increasing pH reduced the carotene content and the chloroplasts became prominent bands.

Table 1. Growth of *T. odorata* in liquid medium at different pH after 6 weeks

pH	Mean increase in diameter of colony (mm)	Mean size of apical cells (μm)*	
		Width	Length
4.5	0.05	9	29 (20-35)
5.5	0.75	4	35(17-46)
6.5	1.10	3	46 (32-58)
7.5	1.20	3	55 (40-70)
8.5	1.15	5	60 (46-70)
9.5	1.15	5	60 (46-70)

*n = 50.

Strength of Culture Medium

Dilution of Bold's medium depressed the growth of the alga, increased its carotene content, and caused a reversion of the characteristic cylindrical forms of actively growing cells to the elliptical shape of cells found naturally on concrete walls (Table 3). At nutrient strengths of 75, 100, and 200%, growth appeared normal, the cells remained green, and their shape was characteristically cylindrical. When the normal concentration (100%) of the

Table 2. Morphological characteristics of *T. odorata* grown in liquid medium at different pH after 6 weeks

pH	Cell shape	Morphological characteristics*		
		Color of filament	Sporangia	Carotene
4.5	elliptical (++) cylindrical (+)	yellow-green	sessile and terminal (++)	(+++)
5.5	elliptical (+) barrel-shaped (++) cylindrical (+)	yellow-green	sessile; terminal and lateral (++)	(+++)
6.5	cylindrical (+++)	yellowish-green (profuse branching)	sessile, usually lateral (+)	(++)
7.5	cylindrical (+++)	light-green	(-)	(++)
8.5	cylindrical (+++)	green	(-)	(+)
9.5	cylindrical (+++)	dark-green	(-)	(-)

*(-) absent; (+) sparse; (++) common/intense; (+++) very common/very intense.

Table 3. Cell shape and colony color of *T. odorata* in different strengths of Bold's liquid medium after 6 weeks

Concentration of Bold's medium	Cell shape*	Colony color
0%	elliptical (++) sub-spherical (+++)	amber
25%	elliptical (++) cylindrical (+)	yellow
50%	barrel-shaped (+) cylindrical (++)	yellow
75%	barrel-shaped (+) cylindrical (++)	yellow-green
100%	cylindrical (+++)	green
200%	cylindrical (+++)	green

*(+), sparse; (++) common; (+++) very common.

medium was doubled, the green colonies consisted of cells packed with chloroplasts, and the presence of carotene was hardly visible. Transferring the alga from the diluted 25 and 50% culture media to normal strength caused the cells to become green within four days and newly formed cells were cylindrical.

Agar Concentration

The mean number of colonies after eight weeks increased from 33 in the liquid medium (0%) to 390 in the medium with 0.7% agar (Table 4). Similarly, the mean number of sporangia formed per colony increased with higher agar concentrations up to 0.7%. At 1-1.5% agar concentrations, when the medium was solid, the mean number of colonies decreased. The mean number of sporangia per colony similarly showed a decline in the 1.3 and 1.5% agar media. At concentrations of 0.1-0.5%, the sporangia were sessile and confined to the central region of the colony while sessile and pedicellate sporangia developed all over the colony at agar concentrations of 0.7% and above.

Growth characteristics also varied with different agar concentrations. In liquid and 0.1% agar media, the colonies were spherical and grew suspended in the medium. At a higher concentration of 0.3%, the colonies ranged from spherical to circular and flat, with the former suspended in the medium and the latter growing on the surface. At concentrations of 0.5% and above, the alga grew only on the surface of the medium with the circular colonies close to one another and the filaments radiating delicately from the center of each colony. In solid media of 1.3 and 1.5%, the circular colonies developed a heterotrichous habit with erect filaments growing from the center.

Table 4. Effects of agar concentrations on the growth of *T. odorata* after 8 weeks

% Agar	State of substratum	Mean no. of colonies	Sporangial no. per colony	Colony shape and formation
0	Liquid	33	8	Spherical, suspended in medium
0.1	Liquid	119	2	Spherical, suspended in medium
0.3	Gel	220	18	Spherical, suspended in medium and circular on surface of medium
0.5	Semi-solid	303	28	Circular and compact, on surface of medium
0.7	Semi-solid	390	87	Circular and compact, on surface of medium
1.0	Firm	14	70	Circular and compact, on surface of medium
1.3	Hard	10	12	Circular and compact, with aerial filaments
1.5	Hard	8	19	Circular and compact, with aerial filaments

Vitamins

The addition of thiamine hydrochloride to liquid medium increased algal wet weight through the enhancement of sporangia] formation which produced more colonies (Table 5). Most of the sporangia were intercalary or terminal in position, d-biotin and vitamin B₁₂ did not affect the growth of the alga. The number of daughter colonies did not increase with the presence of either vitamin in the medium.

The addition of any of the three vitamins did not apparently affect cell size and shape. However, it was noted that d-biotin and Vitamin B₁₂ at the higher concentrations enhanced carotenogenesis since the culture appeared more yellowish than those at lower concentrations. In the absence of vitamins, cultures were green.

Table 5. Effects of thiamine on growth of *T. odorata* after 6 weeks

Thiamine conc. (mg/l)	No. of sporangia	Mean no. of colonies	Mean wet weight of colonies (mg)	Cell length range (μ)*
0	0	25	30.0	26-49(35)
0.1	3	26	43.4	29-46(41)
0.2	10	34	122.2	38-46 (35)
0.3	12	42	140.6	32-46 (38)
0.4	24	76	234.5	41-49(44)
0.5	39	72	154.0	26-41 (35)
0.6	26	48	142.0	38-46(41)

*Figures in parentheses are means of 50 cells measured from the first to fifth cell after the apical cell.

Relative Humidity (RH)

Growth of the colony increased with increasing relative humidities (Table 6). In the absence of moisture in the ambient air or at 0% RH, colonies failed to form new cells nor increase in size, and turned uniformly green after three weeks. Branching of the filaments was sparse and the cells of the peripheral filaments were cylindrically elongated, a shape typical of the alga under liquid culture.

At 35% RH, branches developed from the peripheral filaments. The elliptical cells of the branches contained chloroplasts and numerous small oil globules filled with carotene to impart a light color to the colony (Table 6). At 55% RH, more profuse and longer branches were formed whose cells were longer and cylindrical but subspherical to oval at terminals. At higher humidities, the new cells were cylindrical and much elongated. The yellowish colonies had older central cells packed with carotene-containing

Table 6. Effects of relative humidity (RH) on the growth and morphology of *T. odorata* after 3 weeks

RH(%)	Mean increase in diameter of colony (μ)	Color of colony	Shape of new cells	Sporangial origin
0	0	Green		
32	30	Light yellow	Elliptical	
55	50	Pale green	Elliptical and cylindrical	
81	200	Yellow	Cylindrical	Intercalary
95	250	Yellow	Cylindrical	Intercalary and apical
100	1100	Yellow	Cylindrical	Lateral and apical

oil globules and younger peripheral cells with smaller globules and a less intense carotenoid color. At 95 and 100% RH, most of the apical cells of the peripheral filaments rounded off into sporangia. Many of these apical sporangia were empty, indicating that the zoospores had been liberated as confirmed by the presence of ten new colonies under an RH of 100%.

DISCUSSION

T. odorata is commonly found growing on walls of high-rise buildings in Singapore, especially in areas where there is an excessive runoff of rain water. The presence of the alga imparts an orange tinge to infested surfaces which turn blackish with the presence of blue-green algae. Although *T. odorata* is a green alga it appears orange due to the abundance of carotene which masks the chlorophyll. Carotene is regarded by Senn (1911) as a food reserve since its concentration declines when nutrients are readily available and growth is active. When moisture and nutrients are limiting, the alga accumulates carotene. Also, under shade conditions, the pigment may be completely lacking and the alga appears green (Fritsch 1971).

it has also been suggested that carotene protects the chlorophyll against phyto-oxidation under conditions of high light intensity (Griffiths et al. 1955). Thus, the accumulation of carotene by *T. odorata* growing on walls of buildings enables the alga to survive such an exposed habitat. The ability of the alga to withstand long dry periods also helps in its adaptation to a habitat where moisture is only available during rainy months. The presence of numerous, highly refractile fat granules of various shapes and sizes within the cells would also contribute to the alga's ability to survive drought (Fritsch 1916, Piercy 1917). The complete absence or paucity of large vacuoles in the protoplasm may likewise enable the cell to withstand desiccation (Fritsch

1922). The ability of the cell walls to rapidly absorb atmospheric moisture, as observed in *T. aurea* (Howland 1929), is also another adaptation to dry conditions.

Geitler (1923) earlier pointed out that the appearance of cells of *Trentepohlia* under moisture stress and when nutrients are limiting closely resembles the resting stages of many other algae. This observation is supported by the morphological changes in the cells of *T. odorata* when cultured in mineral nutrients. The shortly stringed elliptical cells give rise to branched filaments of elongated cells, giving the impression of germinating resting cells. The newly formed colony, ball-like in liquid medium and circular in solid, takes a yellow-green appearance as neither carotene nor chlorophyll is the predominant pigment. In liquid culture and under decreasing pH, the cells revert to the natural elliptical shape and have a high carotene content. A reduction in relative humidity resulted in a similar reversion.

In its natural habitat where growth is extremely slow, *T. odorata* is usually sterile. The alga, with its short chain of elliptical cells reproduces vegetatively by cells which readily separate. During periods of rain, detached cells are washed down the surface as indicated by the characteristic vertical orange striations on walls infested with the alga. During dry periods, the detached cells are dispersed by wind as fine dust. Sporangia are rarely formed in nature but, when present, are sessile, terminal, or intercalary in position. In laboratory cultures, sessile and pedicellate sporangia are commonly formed and occur laterally. At pH 5.5 and 4.5 when cells revert to the elliptical shape, the sporangia formed are of the terminal sessile type rarely observed in nature.

The addition of thiamine to the liquid medium also enhances sporangial production, hence, the formation of daughter colonies. However, the production of sporangia and daughter colonies is less than that in a medium with 0.7% agar. The necessity of vitamins for the growth of various groups of algae has been reported. A few members of Cyanophyta are auxotrophs with an absolute requirement for vitamin B₁₂ (Van Baalen 1961). In *Ochromonas*, a Chrysophyta, auxotrophy for various vitamins (thiamine, biotin, B₁₂) is common (Provasoli 1958). Members of the Pyrrophyta, especially the marine dinoflagellates are similarly known to require vitamin B₁₂ (Bold and Wynne 1978). Among the Chaetophorales (Chlorophyta), the first report of a member requiring an external source of vitamin is *Draparnaldiopsis* (Johnstone 1977). The positive response of *T. odorata* to thiamine (but not to biotin and B₁₂) is another record for the Chaetophorales.

The great increase in the number of daughter colonies in a semi-solid medium (with 0.7% agar) can be attributed to the zoospores requiring something solid to attach to prior to germination. These zoospores have been observed to swarm for some time after liberation from the sporangium. They

then come to rest, attach their anterior end to the surface of the glass slide, and rotate for a while before they withdraw their flagella and then germinate. The substratum partly solidified with 0.7% agar is presumably hard enough for the zoospores to attach to and soft enough for them to disperse around, thus providing a larger area for the zoospores to swarm and subsequently get attached prior to germination. When the medium is solid (with 1% agar), the zoospores would not be able to penetrate the surface for swarming.

T. odorata can be cultured easily in the laboratory using Bold's medium either in a liquid or solidified with agar. The alga prefers slightly alkaline (pH 7.5) environment although it can grow within a wide range of pH. This preference for an alkaline environment is not surprising. In nature it colonizes whitewashed building walls as well as painted surfaces where the pH is distinctly alkaline.

The addition of 0.4 ppm thiamine enhanced growth and stimulated the production of sporangia which developed into numerous daughter colonies. Culturing the alga in a semi-soil medium (with 0.7% agar) also promoted the formation of daughter colonies.

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AGROINDUSTRIAL WASTE PRODUCTS AS SOURCES OF CHEAP SUBSTRATES FOR ALGAL SINGLE-CELL PROTEIN PRODUCTION

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ABSTRACT

Four types of agroindustrial waste products were tested for their suitability as substrates for *Chlorella* single-cell protein production. Based on cell density on day 7, unsterilized rice straw filtrate, Tris-buffered inorganic medium (control), unsterilized slop (1%) and unsterilized rice hull filtrate were suitable culture media. Dry weight yield after two weeks did not vary among media. Gross protein content of algae was highest in rice straw with or without sterilization.

Substrates for algal SCP production are assessed on the basis of their nutrient content, pH, and hygienic acceptability.

INTRODUCTION

Mateles & Tannenbaun (1967) in an overview of the status of algal single-cell protein (SCP) production, proposed the establishment of national centers in the United States for studies in the production and utilization of algae. They conceptualized two types of centers operating in tandem, one to work mainly with algal biomass production using inorganic media and hygienic wastes, the other, to serve as model integrated agroindustrial complex that will produce human- and animal-consumable algae and also regenerate and recycle matter for resource and energy conservation. In the Philippines, with the biotechnological thrusts spearheaded by the National Institutes of Biotechnology and Microbiology (BIOTECH) in 1980, a significant boost to algal SCP production was realized. Recently, the United Nations Interim Fund for Science and Technology for Development approved a Philippine proposal to undertake research on microbial SCP

production, utilization, nutrition, and marketing. The organisms under study are the bacteria, yeasts, filamentous fungi, and algae. The combined financial and technological backing provided in the research grants places applied algology, at least at the University of the Philippines at Los Banos (UPLB), on a sustained basis for a minimum of three years. But this is not to say that algal SCP production in the Philippines is in its primordial stage. Noticeable strides during the early seventies include the establishment of cultural requirements, production in synthetic media and animal manure, and feeding experiments involving fish, poultry, and swine (Pantastico & Sulabo 1974; Martinez 1976; Rigor et al. 1980). These past efforts have gone up to the pond production scale.

Chlorella, whose potential as protein feed for swine (Rigor et al. 1980) and as an unconventional protein food for man (Lee et al. 1967) is firmly established, consists of from 50 to 65% protein (Endo & Shirota 1972). Mass cultures of the alga yield from 50 to 65 g (dry weight) per litre of medium under heterotrophic or mixotrophic conditions (Tamiya 1968). Local endeavors, as computed from reports, have realized yields of 0.02 g/l/day in production ponds, reflecting a maximum photosynthetic efficiency of 3.2% (Rigor et al. 1980; Martinez 1980). Evidently, much still awaits algal SCP research in terms of increasing dry matter yield under Philippine conditions.

The presently recognized primary concerns in algal SCP research in the Philippines are the following:

To increase the kinds of test materials as substrates for SCP production with emphasis on those whose product is fit for human consumption;

To establish the optimum cultural conditions with selected media necessary for a pilot-plant production scale;

To expand utilization research after a thorough testing and evaluation of reported processes;

To promote toxicological and epidemiological researches on identified SCP; and

To appraise the marketability of the algal product.

Recognizing the importance of these inextricably linked objectives, we have addressed some of our efforts on the first goal which is to widen the range of test materials for substrates. The preliminary findings here reported involved the use of rice straw, rice hull, molasses, slop, inorganic medium, and Tris-buffered inorganic medium. The general objective is to evaluate the suitability of these materials as sources of algal culture media based on their nutrient content, pH, amount of algal biomass produced, and protein yield of the algal product.

METHODOLOGY

Rice hull and rice straw filtrates were prepared from ashes of the corresponding plant materials. Rice straw, as it is burned by farmers in the field, is a mixture of rice straw and leaves and rice panicle residues. A 10% (w/v) suspension of the ash was prepared with distilled water and passed through coarse filter paper. The pH of the media was adjusted to 9 by addition of 1N KOH. No attempt was made to remove the color of the filtrates, orange-brown in rice hull and bright yellow in rice straw.

Slop and molasses, two by-products of sugar refineries, were separately diluted to 1% concentration by addition of distilled water and their pH adjusted accordingly. For the control, Tris-buffered inorganic medium (pH 9) was used. Aliquots (290 ml) of the various media were dispensed in sterile 500-ml culture bottles and the latter plugged with cotton. Media requiring sterilization were autoclaved at 20 psi for 15 minutes. Chemical analyses of uninoculated media followed involving the following nutrients: total nitrogen by acidimetric method; available phosphorus ($\text{PO}_4^{=}$), stannous chloride method; calcium and magnesium, EDTA titrimetric method. All analyses had two replicates except for nitrogen and phosphorus with three.

Chlorella vulgaris M-3 was obtained from the BIOTECH culture collection at UPLB. Ten-day old cultures were rinsed and suspended in sterile distilled water to make an inoculum of density 12.7×10^6 cells/ml. The final volume of the medium after inoculation was 320 ml.

Cultures were maintained inside a greenhouse where temperature and light conditions fluctuated naturally. They were gently swirled and reoriented twice daily. Data on cell density were obtained at four-day intervals for two weeks using a completely randomized design. All randomly selected replicates of a treatment were discarded after cell density determination. On the 14th day, which followed the last day of cell density determination, cultures were filtered and their dry weight obtained. Then, the total nitrogen and available phosphorus contents of the harvested biomass were determined using the Kjeldahl and molybdovanadophosphoric acid methods, respectively.

RESULTS

Cell density and dry weight

The trends in growth response of *Chlorella vulgaris* M-3 measured in terms of cell density are shown in Fig. 1. Growth was minimal in all types of sterilized media during the first four days. On the other hand, all unsterilized media exhibited better algal growth than did the control. Apparently, *Chlorella* underwent a period of adaptation in sterilized media

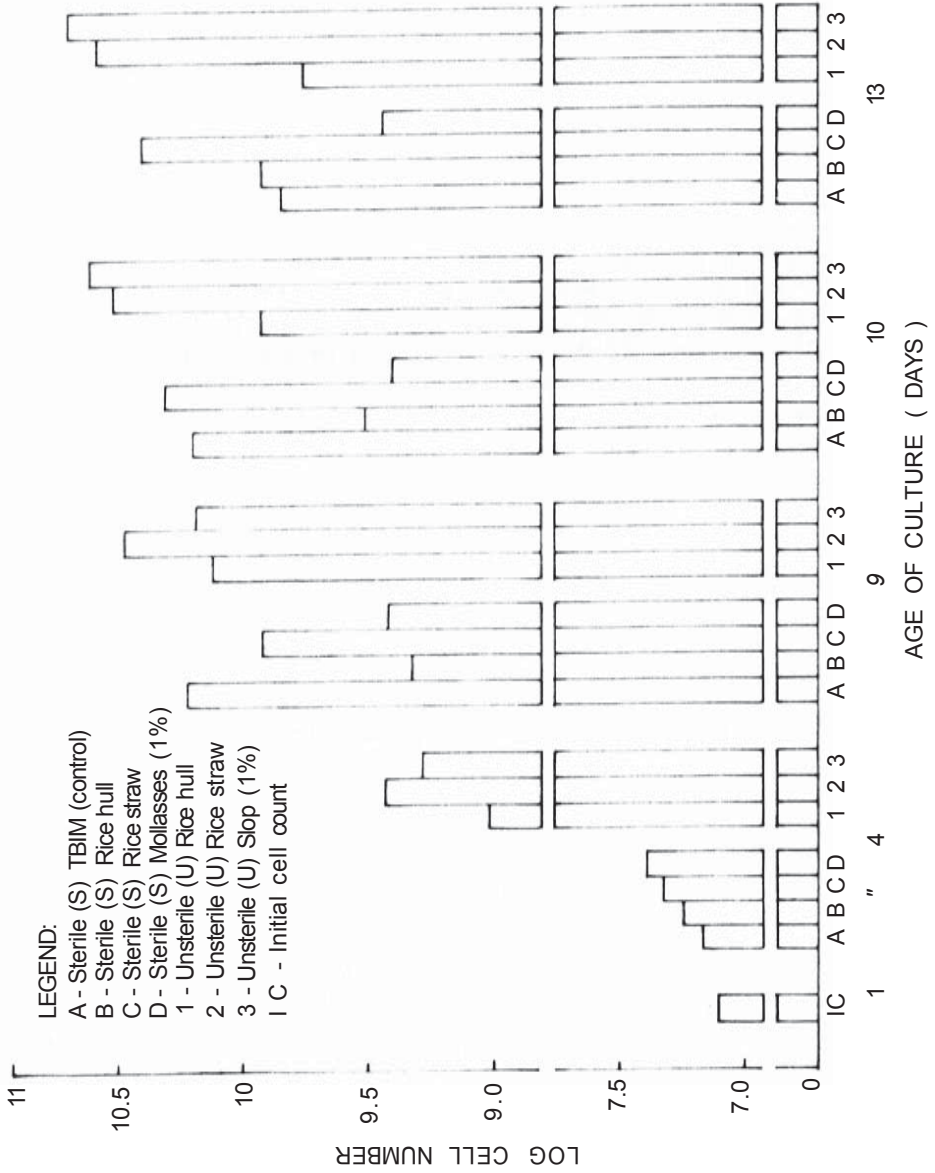


Fig. 1. Trends in growth response of *Chlorella vulgaris* M-3 in different substrates.

during the first four days which was characterized by a pronounced slowing down of growth rate. This was less expressed in unsterilized media.

Indications of substrate suitability became appreciable on day 7. Statistical analyses (Table 1) showed that around the said day, unsterilized rice straw filtrate (30×10^9), TBIM, the control (16×10^9), unsterilized slop (15×10^9), and unsterilized rice hull (13×10^9), in that order, were suitable substrates for *Chlorella* production. Results of Duncan's multiple range test also showed that in general, day 7 is the ideal time for harvest.

The cell doubling time (Stockner & Costella, 1976) of the alga in various media was shortest (7 h) in the control and longest (24 h) in unsterilized slop. An 8-h cell doubling time is normal for the alga (Kingsbury, 1968). Considering the computed averages on day 7, a desired amount of harvest may be achieved at regular intervals if a careful manipulation of the amount of inoculum is made. The possibility of this, however, depends on whether or not the adaptation period of four days as seen in some media could be shortened by preconditioning stock cultures in appropriate media.

The impressive growth behavior of the alga in the slop medium is of particular interest. Compared with molasses, slop has a lighter brown color which does not disappear in two weeks. If this brown color interfered with the degree of illumination of algal cells to an extent that was not determined, then the alga may have compensated for light limitation through heterotrophic growth which is normal in cultures supplied with organic nutrients. Another possible explanation for growth in slop is that it may have supplied certain nutritive substances, possibly organic, that promoted algal multiplication.

Statistical analysis of the dry matter yield (Table 2) showed no difference among the various substrates. This result, which runs counter to

Table 1. Growth of *Chlorella vulgaris* M-3 after seven and thirteen days; cell density at day 0, 0.013×10^9

Media	Cell density ($\times 10^9$)	
	7 days	13 days
Sterilized TBIM (control)	16 ^b	7 ^d
Sterilized molasses	3 ^f	3 ^d
Sterilized rice hull	2 ^d	4 ^d
Sterilized rice straw	9 ^c	24 ^d
Unsterilized slop	15 ^d	51 ^d
Unsterilized rice hull	13 ^{bc}	6 ^d
Unsterilized rice straw	30 ^a	38 ^b

Means with the same letter(s) are not significantly different from each other at 0.05% level, DMRT.

expectations, is attributed to the presence of bacterial contaminants and suspended particles inherent in the media. These interfering substances notwithstanding, it is well to take a cursory look at the dry matter yield data for the purpose of comparing them with those in literature.

Thin-layer (1 cm) cultures of *Chlorella*, considered by Milner et al. (1978) as impractical, yield greater than 50 g dry weight/l/da. The present yields range from 0.008 g/l/da in sterilized rice straw to 0.016 g/l/da in slop and molasses. Assuming the bacterial contaminants to be negligible, the computed biomass closely approximates local harvests from production ponds (0.02 g/l/da) (Rigor et al. 1980; Martinez 1980).

To further evaluate the suitability of the substrates for algal SCP production, the nitrogen content of the resulting biomass was also analyzed (Table 2). Algae grown in sterile or non-sterile rice hull filtrate yielded the highest nitrogen content of from 7.3 to 7.5%, this range exceeding the control by at least 62%. All other yields including that of the control had lower nitrogen content ranging from 3.8 to 5.9%. Multiplying the total nitrogen values by 6.25, the gross protein content was obtained (Table 2). *Chlorella* in rice hull filtrates had roughly 50% protein while the others had from 24 to 37%. The protein content of the former compares favorably with the normal quantities cited by Endo & Shirota (1972) for the alga.

Table 2. Dry matter yield, nitrogen and gross protein contents of *Chlorella vulgaris* M-3 grown in different substrates

Media	Dry matter yield (mg)	N content (%)	Gross protein content (%)
Sterilized TBIM (control)	27.87 ^a	4.5 ^{ab}	25.6
Sterilized molasses	34.83 ^a	3.8 ^{ab}	23.8
Sterilized rice hull	17.90 ^a	7.3 ^{ab}	45.6
Sterilized rice straw	24.80 ^a	5.9 ^{ab}	36.9
Unsterilized slop	35.17 ^a	4.5 ^{ab}	25.6
Unsterilized rice hull	32.66 ^a	7.5 ^{ab}	46.9
Unsterilized rice straw	24.83 ^a	4.1 ^{ab}	25.6

Means with the same letter(s) are not significantly different from each other at 0.05% level (DMRT).

Media Analysis

To explain the above results on cell density and protein content, mineral analysis of the uninoculated media was undertaken (Table 3). The highest level of nitrogen was in unsterilized rice straw filtrate which had an average of 2.91 ppm total nitrogen, roughly eight times that of the control (0.37 ppm). Unsterilized slop and sterilized molasses had moderate levels, 1.87 and 1.59

ppm, respectively, whereas filtrates of unsterilized rice hull, sterilized rice straw and sterilized rice hull together with the control had low levels of 0.99, 0.47, 0.37 ppm, respectively.

With respect to phosphorus, sterilized rice hull filtrate contained the highest amount at 969 ppm followed by unsterilized rice hull and unsterilized rice straw with 813 and 532 ppm, respectively. The P content of slop also exceeded that of the control by over 50%, but molasses is relatively "impoverished" in P with an average of 13.3 ppm. It is quite easy thus to appreciate the fact that rice plant remains are promising materials, nutrient-wise, for use in preparation of media for algal SCP production.

The calcium and magnesium levels of the media were also analyzed. Amounts of these nutrients are similarly abundant (Table 3).

The nutrient content of all media is apparently sizeable, even excessive, for some nutrients. More algal biomass could have been harvested had the density of inoculum been increased. This could have been realized with greater certainty if the algal inoculum had been preconditioned to the appropriate culture medium.

Correlation

Attempts to correlate cell density with the independent variables revealed two interesting relationships: nitrogen and pH positively correlated with cell density. The relationship with respect to N suggests that this nutrient may have limited algal response to various media. The proportion of P to N is tremendously lopsided (Table 3).

The positive correlation between pH and cell density on day 7 invites attention. Among the media, rice straw with or without sterilization underwent the least departure from its initial pH of 9. Unsterilized slop

Table 3. Initial levels of nutrients and final pH of different substrates

Media	Nutrient concentration (ppm)				
	N	P	Ca	Mg	pH
Sterilized TBIM (control)	0.37 ^c	177.7 ^c	182 ^c	179.8 ^{ab}	10.0 ^a
Sterilized molasses	1.59 ^b	13.3 ^f	683 ^a	179.8 ^{ab}	4.5 ^f
Sterilized rice hull	0.37 ^c	968.7 ^a	78 ^c	212.2 ^a	7.6 ^d
Sterilized rice straw	0.47 ^c	532.0 ^c	117 ^c	185.0 ^{ab}	9.3 ^b
Unsterilized slop	1.87 ^b	230.7 ^c	455 ^b	95.6 ^{ab}	8.4 ^c
Unsterilized rice hull	0.99 ^{bc}	812.7 ^b	455 ^b	41.7 ^b	7.3 ^e
Unsterilized rice straw	2.91 ^a	417.7 ^d	390 ^b	38.6 ^b	9.5 ^o

Means with the same letters) are not significantly different from each other at 0.05% level (DMRT).

followed with pH 8.4. Rice hull dropped to around neutral, pH 7.5, while molasses became acidic with pH 4.5. The control became more alkaline, pH 10 (Table 3). These findings seem to indicate that in addition to the combined growth effects of undetermined factors in the medium, the pH factor exerts an appreciable effect on the ability of algal cells to multiply. Thus among the media, rice straw filtrate elicited better growth probably because it retains a pH level closest to that preferred by *Chlorella*, around pH 9. The same reasoning probably holds for the slop medium. There is also the possibility that unchecked pH levels had pronounced effects upon 2-week old cultures. These results emphasize the importance of a sustained pH optimum to ensure maximum sustainable yield.

DISCUSSION

The current trends in human population growth reiterate the underscored needs of the past decade for the formulation of policies that would solve the food, energy, and population problems. Single-cell proteins have time and again been turned to, reportedly as unconventional protein sources. Setting aside the issue of individual taste preferences and cultural taboos on food, it is incumbent upon the SCP researcher to recommend foodstuffs like algae which are grown under hygienic conditions. Not only must the culture be hygienically acceptable by the best of human standards; it also must generate the minimum of affront to human senses. Domestic and animal wastes clearly hold a lot of promise in terms of their ability to generate biomass fit enough for farm animal consumption. But knowing the vast areas required to meet the necessary production rate, it is not difficult to imagine the degree of pollution of the air over the production site involving animal wastes.

Rice straw and rice hull as sources of culture media have a good potential for use in algal SCP production. They are hygienic substrates in that they do not lead to fouling of the air. They have the following advantages, namely, 1) cheapness, 2) availability, 3) simplicity of preparation, 4) nutrient sufficiency, 5) pH adequacy, 6) potentially high protein yield of the algal product, and 7) recycleable residues. Slop is another medium with a high nutrient content. A cheap hygienic substrate, it has the consistency and taste of molasses, not to mention its appetizing aroma. This substrate also enables heterotrophic growth of the algal SCP, supplying as it does the energy and carbon dioxide requirements of the culture organism.

Preliminary as these findings are, some important thrusts of immediate efforts in algal SCP production become identifiable. First is the need to establish the optimum concentrations of the plant filtrates and slop used. Second is the need to undertake a complete nutrient analysis of the media in order to determine what nutrient enrichment procedures are necessary.

Residual nutrients do have to be determined to establish the optimum amount of the inoculum. Third is the need to determine what size of inoculum is optimum for the highest sustainable yield to be realized. Fourth is the need to establish what culture maintenance procedure to undertake for maximum sustainable yield. Lastly, the need is felt for a systematic enumeration of microorganisms that contaminate the algal cultures. It is only after these immediate problems shall have been solved that one may embark on the formulation of algal food preparations fit for human consumption. The next few years will see renewed efforts to accomplish these goals.

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UTILIZATION OF SEAWEED RESOURCES

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SEAWEEDS AS SOURCES OF INDUSTRIAL GUMS

The commercial importance of seaweeds derives principally from their use as sources of industrial gums such as agar, carrageenan, and alginic acid. These gums are made up of the structural polysaccharide material found in red seaweeds in the case of agar and carrageenan, and in brown seaweeds in the case of alginic acid. Such gums have many industry applications.

Seaweed gums are unique in that they impart to various processed products special physical properties relating to viscosity, texture, and gelling ability. These properties are dictated by the chemical structure of the polysaccharide. Seaweed gums are made up of unique sugar molecules. Agar is made up of repeating units of *B*-D-galactose and 3,6-anhydro-*B*-L-galactose; carrageenan of *B*-D-galactose and 3,6-anhydro-*B*-D-galactose sulfated at various positions depending on the type of carrageenan; and alginic acid of mannuronic and guluronic acid residues. These different polysaccharidal structures can be distinguished from one another by means of their infrared (IR) spectra. Each spectrum is essentially a "fingerprint" of the polysaccharide. Thus, the spectrum of agar is distinct from that of carrageenan or alginic acid. A spectrum arises as a result of vibrations unique to each kind of molecule. There are peaks that are unique to each polysaccharide and which are indicative of certain structural features present in the molecule, e.g., the 3,6-anhydro galactose moiety gives rise to the peak at 930 cm^{-1} ; the various ester sulfate groups to peaks between 800 and 850 cm^{-1} .

Seaweed polysaccharides are extracted by various processes. Common to all is the extraction of the polysaccharides into water. This indicates that seaweed gums are fairly soluble in water, a property that is again dictated by molecular forces arising from the preponderance of such groups as -OH (hydroxy), -OSO₃- (sulfate ester) and -COO (carboxylate) in the macromolecule. In the Philippines, *Eucheuma* is presently the most important source of carrageenan. *Eucheuma alvarezii* yields kappa carrageenan while *E. denticulatum* yields the iota form. There are, however, other carrageenan-containing seaweeds that can be found in tropical waters. The carrageenan isolated from *Acanthophora* appears to be of the lambda form, while that

from *Hypnea* appears to be a variant of kappa carrageenan. The cultivation of these seaweeds is a matter that should be further investigated as these are sources of carrageenans that are of great commercial importance. The carrageenan from *Hypnea* can form very strong gels; that from *Acanthophora* possibly can be used as a stabilizing agent in non-viscous liquid products.

The extraction process for agar involves a freezing-thawing process. *Gracilaria verrucosa* appears to be the most promising species. However, other species also have potential economic value, at least in the production of food-grade if not bacteriological-quality agar.

The extraction process for alginate essentially involves the conversion of naturally-occurring alginate into its soluble form, i.e., sodium alginate. Most, if not all, of the alginates that can be bought in the world market today come from kelp. However, there are tropical seaweeds that are potential sources. *Sargassum* appears to be most promising.

CARRAGEENAN AS SUBSTITUTE FOR MICROBIOLOGICAL AGAR

Recent investigations on the use of carrageenan in microbiological media show that this application is indeed feasible. Carrageenan processed from *Eucheuma* has been especially formulated and tested as a medium for the growth of a wide spectrum of microorganisms - bacteria, yeast, and other fungi. The carrageenan medium has been tested in a number of research and teaching laboratories and satisfactory results have been obtained. The use of carrageenan as a substitute for bacteriological agar may be a boon to developing countries such as the Philippines where the rising cost of agar has been most strongly felt.

SEAWEEDES AS BINDERS OF HEAVY METAL POLLUTANTS

The use of seaweeds as a pollution-control agent has also been investigated at some length. Basic chemical studies conducted to date show that such an application is feasible. The approach used in the study consists of the following methodologies: 1) dialysis of solution of pure seaweed polysaccharides against solutions of heavy metal salts; and 2) elution of heavy metal salt solutions through a column of ground, dried seaweed. The metals that have been investigated are lead, cadmium, copper, zinc, iron, and mercury. The affinities of both carrageenan and alginate for these metals have been examined. Both polysaccharides exhibit a preferential affinity for lead, although the affinity for the other metals are not insignificant. The affinity for copper, in fact, almost matches the affinity for lead. *Sargassum* is

presently being developed for this particular application because of its ease of handling. Preliminary experiments with actual industrial effluents contaminated with lead and cadmium indicate that an industrial system for wastewater treatment that uses *Sargassum* as metal binder can be developed.

OTHER POTENTIAL SEAWEED APPLICATIONS

Although the use of seaweeds as fertilizer in agriculture and horticulture has been introduced on a commercial scale in some European countries, this particular application still has to be developed in Asian countries. There are reports that some coastal communities in the Philippines do use *Sargassum* occasionally as a fertilizer. However, scientific studies that will lead to its more widespread use have been limited. Pre-development studies involved chemical studies on auxin-like substances from *Sargassum polycystum*. Auxins are plant growth hormones and their presence in *Sargassum* may partly account for the fertilizing property of this seaweed. Substances which exhibit auxin-like activity have indeed been isolated and partially characterized. Chemical formulas have been obtained although their exact identities have not yet been established. None of the compounds isolated were found to be indolic in nature (most known auxins possess the indole group).

The use of seaweeds as food is not as widespread in the Philippines as it is in Asian countries, particularly Japan. A study that looks into the nutritive value of some edible Philippine seaweeds is presently being undertaken. Nutritive value is being analyzed in terms of crude protein content, amino acid composition, and mineral and vitamin content. Results to date indicate that seaweeds are not the best sources of protein. However, they are excellent sources of minerals and do contain some vitamins.

CULTURE AND UTILIZATION OF FRESHWATER ALGAE AS PROTEIN SOURCE

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INTRODUCTION

Mankind is faced with great challenges in the years ahead. The future prospects would include an even higher incidence of hunger, starvation, and malnutrition. The production of food from unconventional sources may alleviate some of these problems. It is predicted that the earth's population will increase by at least 50% to a total of 6 billion by the end of the century. It is expected to double to 8 billion in the 21st century. Large cities such as Mexico City, Sao Paulo, and Calcutta will have population of 30,26, and 16 million, respectively, by the year 2000 (Blume 1979).

With the world's increasing population, large amounts of protein will be required for human and animal feed (Meadows et al. 1972). The protein deficit is markedly seen in many Third World countries and has led to massive investments in research on single-cell (microbial) protein (SCP). However, it is desirable that SCP be produced in a very large scale and with the cheapest available substrates (Meyer 1980).

UTILIZATION OF ALGAE FOR INDUSTRIAL PHOTOSYNTHESIS

The scientific and economic evaluation for the commercial production of algae in mass culture on inorganic media has been thoroughly studied by different groups. A comprehensive survey of the relevant work published was compiled by the Carnegie Institution of Washington (Burlew 1953). Tamiya (1957) gave a detailed review of the technical aspects of mass culture of algae and found reason to be moderately optimistic about the commercial future of the process.

With the advances in algal culture, three lines of development are distinguished: (1) culture of algae for the production of useful organic materials as food, feed, and some special organic materials; (2) culture of

nitrogen-fixing algae for increasing soil fertility; and (3) culture of sewage algae in symbiosis with bacteria with a two-fold purpose - to accelerate stabilization of organic sewage material, and to utilize the harvested algae as in (1).

The culture techniques and species of algae are more or less different. In (1), fast-growing green algae such as *Chlorella* and *Scenedesmus*; in (2), blue-green algae which are strong nitrogen fixers like *Nostoc*, *Anabaena*, *Tolypothrix*, etc.; and (3), mixed cultures of various algal strains in harmony with sewage bacteria.

Although different in aims, these projects have many common problems related to the physiology and biochemistry of algae under carefully controlled laboratory conditions. The main physiological characteristics of the algae involves the variety and flexibility of their nutrient requirements and the chemical composition of their cells.

Pioneering investigations on large-scale culture of algae under controlled conditions in inorganic media were conducted in the US, Germany, and Japan in the 1950s. In the 60s, cultivation of algae was investigated as a means of bioregeneration of wastes in chemocycle systems for extended space exploration missions or in treatment of wastes in sewage oxidation ponds (Oswald & Golueke 1968). More recently, algae were again considered for producing protein as food or feed in developing countries (Durand-Chastel & Clement 1975).

Considered algal pilot plant operations were those conducted at the University of California; Czechoslovakia; the Indian/West German System in Mysore, India; the Sosa Texcoco Process in Mexico City; that in Technion, Haifa, Israel; and in Kyoto University in Japan.

SOCIOECONOMIC IMPLICATION OF ALGAL MASS CULTURE

Out of about 17,000 algal species that have been described since the turn of the last century, only a few have been investigated and described as excellent for possible sources of protein. Among the important ones are species of *Chlorella* (*C. pyrenoidosa*, *C. vulgaris*, *C. ellipsoidea*), *Scenedesmus*, and the nitrogen-fixing blue-green algae (*Anabaena variabilis*, *A. cylindrica*, *Nostoc commune*, *N. muscorum*, *N. punctiforme*, *Phormidium molle*, *Tolypothrix*, *Stigonema*, *Nodularia*).

The fertility of rice fields in Southeast Asia and the Malay Archipelago is said to depend in some measure on the nitrogen-fixing species of *Tolypothrix*. In India, mixed cultures of *Aulosira* and *Cylindrospermum* are used.

Chlorella has been studied in great detail due to its high rate of photosynthesis, carbon dioxide consumption and release of equimolar quantity of oxygen, and high content of protein (40-60%, dry weight). That *Chlorella* could be used in a bioregenerative life support system of a space craft implies that it could serve also as an important source of food.

Detailed data on the chemical composition in terms of assimilation and biological value of *Chlorella* and *Scenedesmus* have been reported and discussed (Table 1) (Cook 1962). Assimilation of *Chlorella* and *Scenedesmus* mixture (1:10) was insignificantly bettered by boiling and autoclaving.

In recent years, people in certain countries (e.g., Taiwan) have incorporated relatively small amounts of extracted algal cells in their diet. Unextracted algae produced a very disagreeable flavor when used as food ingredient. However, Hayami et al. (1969) found that Japanese women would accept 30 grams methanol-extracted algae per day in their diet. A drawback here is that the green color of algae is difficult to mask in most food mixtures.

The cell walls of algae are fibrous and can irritate the gastrointestinal tract. Algae used in Taiwan are steam-treated to rupture the cell walls and release the cell content. As much as 150 g of methanol-extracted algae per day could be consumed without complaints. The estimate of the nutritive value of *Chlorella* and *Scenedesmus* abated the original proposal on their utilization for human consumption and directed further studies along two lines: (1) examination of the practicability of using *Chlorella* and *Scenedesmus* as animal feed, and (2) preparation from the algae biomass of protein concentrates for human consumption.

However, difficulties involved in protein extraction, poor food properties and costly technological processes suggest that the preparation of protein products from *Chlorella* and *Scenedesmus* is not yet a primary and beneficial solution to the protein problem.

A study of the protoplast of various algae may give the key to a greater use of algal protein. The single-celled *Dunaliella* and *Cosmarium* whose protoplasts may become a potential protein source have been described. Interesting reports on the preparation of *Chlorella* protoplasts by means of

Table 1. Biological value of algae (After Cook 1962)

Protein Source	Biological Value
Dry algae	54.2 ± 3.2
Autoclaved algae	55.5 ± 2.6
Algae boiled for 30 min.	56.0 ± 2.7
Algae boiled for 180 min.	48.7 ± 2.6

hydrolytic enzymes had been published (Gibbs & Dorffres 1976; Berlines & Wenc 1976; Bruan & Aach 1975).

Of particular interest is the unicellular blue-green alga *Spirulina*. This alga is relatively large (about 100 times longer than *Chlorella*). There are good grounds for believing that it may be well suited for human consumption because the Africans on the shores of Lake Chad consume it as part of their diet (Table 2). The alga was also eaten by ancient Aztecs in Mexico.

Table 2. Chemical analysis of algal cakes on sale in the Republic of Chad (Azoulay & Senez 1960; Champynot 1965)

Protein	45-46%
Fats	5-6%
Carbohydrates	16-20%

Experiments to assess *Spirulina* assimilation have been conducted in France and Mexico (Table 3) (Clement et al. 1968). The results were so convincing that the Mexican government permitted the sale of *Spirulina* in 1973 (Clement 1975).

Table 3. Chemical analysis of *Spirulina* (Clement et al. 1968)

Protein	62-65%
Pats	2-3%
Carbohydrates	18-20%

The value of traditional recycling methods used in rural areas of Asia is also being increasingly widely recognized. An example is the Indonesian village pond in which wastes are converted to algae which are then eaten by fish. Lately, with the energy crisis in the 70s, the Asian Pacific countries got involved in a technique which started out as a means of waste utilization and opened the way to bioenergy through aquaculture on any scale desired. In principle, one and the same stock of mineral nutrients can maintain a constant supply of algae and methane. At present, capacities of 80 tons dry weight per hectare per year have been obtained but a further increase is still expected. These agricultural practices of recycling minerals by waste conversion to useful production opened up a new outlook toward aquafarming by rendering land more productive and making these minute plants accessible for human use.

FOOD SITUATION IN THE PHILIPPINES

The Filipinos are suffering from a deficiency of protein since our average daily protein intake of 42.5 g is far below our daily requirement of 68 g. Our total production of high protein foods seems barely sufficient to supply 50%

of our protein needs. Even with all our importation of high protein foods such as eggs, milk, fish and meat products and our eating of low-protein foods, our deficiency in protein is still enormous. If we add to this picture the fact that our population is increasing very fast and that to cope with this situation we have to constantly extend land area under cultivation, the thought naturally occur that a time will come when our fertile land will be limited by crowded population and could no longer supply our need for food. As a means of alleviating the threat of famine, particularly protein famine in this country, we should also explore the culturing of high-protein food algae not only for human but also for fish and animal nutrition.

The Philippines, being a tropical country, is naturally rich in algal flora. In raising high-protein algal food it is advantageous to use our local species as they are already accustomed to living under the existing local conditions, and therefore, their culture may not necessitate drastic modifications and adjustments of these conditions in order to favor their development. Strains which can tolerate high temperature or which may not be affected by diversified environment can be used. With such algae, the use of a cooling device similar to that used in culturing algae in temperature countries may be obviated.

NIST'S RESEARCHES ON ALGAL CULTURE

Early investigation was conducted in 1954 at the Biological Research Center, Institute of Science and Technology to explore the algal flora of Manila and nearby provinces for high-protein strains of unicellular green algae. The exploration yielded several *Chlorella* isolates in pure state. A detailed study of a select isolate, *C. pyremoidosa* Chick was made (Palo et al., 1965). This alga was found to contain more than 60% protein (dry weight basis), with all the essential amino acids and vitamins present; very prolific in growth (rate of more than 33 times the initial cell number in 4 days); and tolerate high temperatures (up to 46°C), fully exposed to sunlight during summer.

Later, further work was continued on four local *Chlorella* strains. The effects of media with low and high available nitrogen on the protein and lipid contents of the algae were determined (Rodulfo et al. 1972). The results were in agreement with those of Milner (1948, 1951) who showed that the chemical composition of *Chlorella* "can change in response to change in environment." Harvests with as high as 36.4% lipid and 22.7% fat were obtained by this method: The methanol extracts of *Chlorella* were active against *Micrococcus aureus*, *Bacillus cereus* and *B. subtilis*. This confirmed the presence of some antibiotic substances in their cells. While the culture of algae did not reach pilot plant scale level at NIST, stock cultures of *Chlorella* were made available to other research institutions and to institu-

tions of higher learning, fish culturists, etc. who would like to venture in algal culture.

Some other researches done at NIST were on *Scenedesmus obliquus*, another fast growing green alga which is equally rich in protein (50%, dry weight). A comparative study on bench-scale production of *Chlorella* and *Scenedesmus* was made for a year. Environmental conditions such as those of temperature and light were not controlled. Results showed growth of these algae to be favorable at summer time although proliferation of rotifers and other protozoa could not be avoided.

In the 70s, the NIST pursued further research on cultivation of the nitrogen-fixing blue-green algae with emphasis on edible forms commonly utilized by natives in Northern Luzon. One filamentous edible species, *Nostoc linckia*, locally called *tabtaba* was studied (Rodulfo 1980). This alga grew best in a nitrogen-free inorganic medium, had a protein content of 40-45% (dry weight), and grew well in an alkaline pH (7.5-8.0). Another very interesting species, *Nostoc commune*, was isolated from Albay in the Bicol region. The richness of the soil in the region could be due to the presence of this alga which was very prolific in the area. Growth was better in a nitrogen-free solution at pH 6.8-7.2. Maximum increase in colony size was 2.5 cm after two weeks (from initial pin size). Blue-green algae promise to be a very good source of protein fitted for human consumption.

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PHILIPPINE ALGAL TAXONOMY: PAST, PRESENT, AND FUTURE

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ABSTRACT

This paper presents a historical account of the development of algal taxonomy in the Philippines, from its early beginnings in 1800 to the present, with emphasis on marine forms.

Marine algal taxonomists in the country are urged to shift emphasis from the classical morphologic approach to the chemotaxonomic and cytologic method in attempts at resolving the classification and phylogeny of important marine groups such as the polymorphic and economically important *Caulerpa*, *Ulva*, *Codium*, *Sargassum*, and *Gracilaria*. Chemotaxonomy has close affinity with the morphological approach, hence is given priority over cytology with the use of the scanning electron microscope.

INTRODUCTION

This paper attempts to present an overview of the status and future direction of algal taxonomy in the Philippines, with emphasis on marine algae. This is in recognition of the significant role the taxonomists play in providing baseline data that link them with other scientists.

A developing country such as the Philippines expects to benefit from this symposium on the culture and utilization of algae. The information to be gained will constitute a significant addendum to the rather anemic algal literature of the country.

HISTORICAL ACCOUNT OF ALGAL TAXONOMY

Available literature and actual explorations show that the Philippines is rich in algal materials for biological laboratory studies as well as for

commercial utilization. Most of the early studies were taxonomic works by foreign or visiting scientists.

1800-1900

During the early nineteenth century, various foreign expeditions as well as individuals undertook botanical collecting in the Philippines.

A. von Chamisso, botanist of the Romanzoff Expedition (1817-1818), collected the first *Corallopsis* specimen in Manila. The Philippines was not in the itinerary of this Russian exploration, but the ship *Rurik* was forced to take shelter in Manila following a heavy storm in the Pacific.

C.A. Agardh (1820) described and illustrated the type species under the name *Sphaerococcus salicornia* which was later assigned to *Corallopsis* and more recently to *Gracilaria*. R. Greville (1830) monographed *Corallopsis* based on the morphology of Chamisso's Manila material.

The Prussian East Asia Expedition headed by F.J.E. Meyen visited the Philippines in 1831. From among the materials collected in Rizal and Laguna, Georg von Martens found two new *Cladophora* species, the freshwater *Cladophora diluta* and *C. luzoniensis*.

The year 1837 might be considered the birth of algology as a science in the Philippines. It was then that the earliest mention of the algae of the country was made by Blanco (1837) in his book *Flora de Filipinos*. Two more editions of the book followed, one in 1845 by Blanco himself, and a posthumous edition in 1877-1883, completed by Fr. Ignacio Mercado and Fr. Antonio Llanos. Blanco revised a number of algal identifications he himself had made. The scarcity of botanical literature at the time and the limited academic contact with foreign algal taxonomists caused some misidentifications and duplications of scientific names.

A Manila-based Londoner, Hugh Cumings, made some valuable collections of algae that were believed worked on by Montagne (1844-1846). A great number of the Cumings collections are deposited in the Kew Herbarium in England.

An American expedition in 1854 collected mostly flowering plants and a few algae; there was no algologist in the group. The algal specimens were turned over to Bailey and Harvey who reported new records like *Dictyota dichotoma* and a host of new species, among them *Amphitetras favosa*, *Campylodiscus kutzingii*, *Lagena williamsonii* and *Triceratium orientale*. The collecting site, as reported by Charles Wilkes, head of the exploration, was confined to Marongas Island, northeast of Jolo in southern Philippines.

Georg von Martens (1866), in his compilation of algae described or

reported from tropical Asia and the Pacific, made some revisions of the nomenclature in Blanco's *Flora de Filipinos*, e.g., *Fucus gulaman* Blanco, renamed *Fucus edulis*, to *Sphaerococcus gelatinus* Agardh.

After the British Challenger Expedition (1874-1875), Dickie (1876, 1877) cited in his enumeration *Polyphysa spicata* Kutz. from Mactan Island in the Visayas.

In 1919-1921, Shaw published his exceptional study of new volvocine genera: *Campbellosphaeria*, *Janetosphaeria*, *Merrillosphaeria*, and *Copelandosphaeria*. Shaw (1923) also reported *M. africana* from Manila.

The last recorded exploration that reached the Philippines in 1800-1900 was that of the Italian *Vettor Pisani*. The collection yielded new species as described by Piccone (1886).

1900-1941

The year 1900 saw the coming of the Dutch Siboga Expedition which undertook intensive dredging in the Sulu Sea. The specimens were studied by different investigators. Van Bosse & Foslie worked on the coralline group; Barton on *Halimeda*; A. & E.S. Gepp (1911) on Codiaceae. Weber van Bosse (1913-1928) published in two parts her annotated listing of blue-green, green, brown, and red algae. She made special mention of the abundance of *Bornetella sphaerica* (Zanard) Solms-Lauback. The bulk of the collection was preserved supposedly in Holland and elsewhere in Europe. Unlike previous expeditions, the Italian group failed to turn over duplicate materials to the Philippine Government.

Between 1907 and 1910, the United States Fish Commission boat *Albatross* visited the Philippines and some collecting was done. The Chlorophyceae was partly worked out by Gilbert (1941, 1942a,b, 1946, 1947). Velasquez (1963) cited most of the blue-greens years later.

In 1913, Merrill and Shaw sent to the United States some marine algae now deposited in the New York Botanical Garden. Most of the green algae were loaned to and studied by Gilbert who described a new species, *Acetabularia philippinensis*.

Collection trips that followed were rather small-scale joint ventures with the Philippine Government. Outstanding were those by Bartlett on two occasions. In 1935, Bartlett, who at that time was with the University of the Philippines as exchange professor, undertook extensive algal collecting from Batanes down to Sulu. His collections were sent to the University of Michigan.

Manza (1937a-c), a Filipino marine botanist, worked on articulated

coralline algae and described such new genera as *Bossea* and *Joculator* as well as some new species.

Bartlett made his second visit to the Philippines in 1940-41 as a full-time agronomist. Nevertheless, he included seaweeds in his collections, with the assistance of J.S. Domantay, then with the Bureau of Fisheries, and a Filipino Muslim diver. The second batch of marine algal specimens was also sent to the University of Michigan and identified and distributed under the supervision of W.R. Taylor. The chlorophyceans appeared in the papers of Gilbert (1941, 1942a,b, 1946, 1947); phaeophyceans in Taylor's (1961, 1962, 1963, 1966); myxophyceans in Velasquez's (1940, 1941a,b, 1962).

1942-1945

The outbreak of World War II set back algological study in the Philippines. The country, scene of some of the world's fiercely fought battles, saw its herbarium, then under the Bureau of Science, reduced to ashes during the liberation of Manila. Nothing could be salvaged from the debris; algal and flowering plant materials and valuable references disappeared in the flames.

A reported study during the period was that of Dawson (1954) who collected *Corrallopsis salicornia* along the sea wall of the Manila Harbor. His material later became the topotype of the present *Gracilaria salicornia* (C. Agardh) Dawson and, therefore, confirmed the real type locality of Chamisso's erstwhile *Corrallopsis* material which was doubted by Ruprecht in 1851.

1956 to the present

Rebuilding Philippine phycology was a very challenging task. The initial step was taken by Velasquez who, under a grant-in-aid program from the American Philosophical Society, continued his research on the myxophyceae. A number of his masteral students assisted in the rebirth of research and accumulation of references on algae.

The next step was the organization of the Phycological Society of the Philippines under the initiative of Dr. Velasquez. The aim was to build a "bank" of well-duplicated numbered specimens, only roughly identified as to family or genus, from which specialists might receive materials for use in monographic or regional studies.

In November 1964, one of the biggest post-war expeditions arrived in the Philippines. This was the joint exploration-collection of the Kagoshima University of Japan and the National Museum of the Philippines. The places of collection were the islands of Batan, Batanes, and Camiguin, and the provinces of Cagayan and La Union (San Fernando), all in the northern part of the country. The expedition lasted for one month and yielded a very

substantial] number of algal materials including freshwater forms. Tanaka (1967) described some new species out of the collected materials, like *Avrainvillea capituliformis* and *Dictyopteris camiguensis* from San Pioquinto, Camiguin Island, Cagayan Province, and *Claudea batanensis* from Basco, Batan Island, Batanes Province.

No notable expedition has been undertaken after the 1964 joint exploration. However, the University of the Philippines, through Dr. Velasquez and his students, has organized collection trips aboard the university training ship *Pampano*. The Philippine National Herbarium has had its share of algal collection trips through the initiative of the author and his co-workers in the Division of Botany.

The Philippine National Herbarium shortly after the war was transferred to the National Museum. The algal section was left with no one to restore the precious specimens that formed part of one of the richest herbaria in the world. Except for few duplicates recovered from foreign herbaria, very few materials were added. Numerous marine forms from various rich and previously unrepresented collecting grounds have been accumulated since 1963. The Philippine National Herbarium today boasts of algal specimens from different parts of the country as well as of foreign duplicates kept as exchange materials.

The 60s witnessed the turning point in algal taxonomy in the Philippines. It marked the initial active participation of Filipino phycologists in taxonomic studies especially those dealing with marine algae.

CURRENT STATUS

The algae of the Philippines are probably the best known taxonomically in the tropical Pacific. Earlier works have already been mentioned above. Cordero (1977) studied the red algae, while the freshwater plankton became the subject of research by Pantastico (1977) and Martinez & Eakle (1977). The brown algae group is being studied by R. Modelo, Jr. at Kyoto University. Contribution by Dr. G. Trono, Jr. and his students at the University of the Philippines and by Dr. E. Menez of the Smithsonian Institution (including his students at Siliman University and University of San Carlos) also advanced significantly marine biology research in the country.

Velasquez et al. (1975) listed 229 genera and 824 species for the Philippines based on 88 publications, the earliest by Rumphius (1750) and the latest by Cordero (1977).

No less than 20 genera contain several species considered as potentially of economic importance. Some of these are the green *Enteromorpha*, *Ulva*,

Caulerpa, *Codium*, *Monostroma*, and *Chaetomorpha*; the brown *Hydroclathrus* and *Sargassum*; and the red *Asparagopsis*, *Acanthophora*, *Eucheuma*, *Gelidiella*, *Gracilaria*, *Hypnea*, *Laurencia*, and *Porphyra*.

Caulerpa, *Sargassum*, *Eucheuma*, *Gracilaria*, and *Porphyra* promise to yield economic benefit once maricultured intensively. Except for the Philippine species of *Sargassum*, the taxonomy of species in these genera has been worked out substantially by both foreign and Filipino phycologists.

To date, only *Caulerpa racemosa*, *Eucheuma striatum*, *E. spinosum*, and *Gracilaria verrucosa* have been farmed vegetatively using cuttings. There were previous attempts in 1980 to mariculture *Porphyra* by the Bureau of Fisheries and Aquatic Resources (BFAR), but with little success due to unsustainable funding. *Porphyra* in the country is expensive, its supply being dependent only on natural growth in the coastal waters of northern Luzon. It promises to be a dollar-earning marine commodity. Three species occur in the country (Cordero 1974, 1976), namely, *P. crispata* Kjellman, *P. suborbiculata* Kjellman, and *P. marcosii* Cordero. The latter two are recommended for sea farming.

FUTURE DIRECTION

The paucity of algal taxonomists in the Philippines is very pronounced. This becomes even more apparent when we consider that the phycologists in the country are based in Luzon. The Philippine Archipelago, with its 7,100 islands and a coastal line more extensive than that of continental U.S.A., is a virtual paradise for phycologists. It is, however, a difficult place to work in. Distance between islands has to be reckoned, logistic support is often entirely self-borne, and the security in some areas is unstable.

It was only in the late 70s when phycology graduates started to proliferate. Before, taxonomy and most biological sciences were taboo to Filipino students. As one writer puts it, taxonomy is not a "fashionable" science; even in terms of funding it plays second fiddle to its sister sciences like physiology and ecology. There was also the misinformed notion that taxonomy is highly specialized, and the dim chance of getting a good-paying job was most feared by students. There is some truth to this because taxonomy contends with the difficult problem of classifying biological organisms. Still, the problem involving shortage of manpower adept in algal taxonomy and absence of a well-defined national algal research program must somehow be alleviated soon.

For the present, the Philippines needs a redirection of its approach to algal taxonomy from the present classical method. This is not to say that we do away with the latter. Rather, we recognize the classical method as

integral part of the taxonomic scheme, but we must gradually shift to the cytological approach using the scanning electron microscope (SEM). However, considering the prohibitive cost of the SEM it is advisable for Filipino algal taxonomists to give more emphasis on chemotaxonomy as a tool toward improving taxonomic output. Chemotaxonomy has a close affinity with the classical morphological method. It requires team effort - a histochemist, someone to do immuno-electrophoresis, and another to do spectroscopic analysis of polysaccharides, proteins, and other pigments in the cytoplasm and cell wall of algae.

We are now doing the chemotaxonomy of brown algae in one area in Luzon. Later, we hope to resolve with the same approach some fundamental problems in the taxonomy of such economically important genera like *Ulva*, *Caulerpa*, *Gracilaria* and *Sargassum*. For these genera, life-history studies are also needed. *Sargassum* needs utmost attention. Velasquez et al. (1975) list 27 species for the country, but the veracity of this record is difficult to ascertain in the absence of a more intensive taxonomic study of the genus, itself highly polymorphic. *Sargassum* is the country's answer to the algin-rich kelp (*Macrocystis*) of temperate waters.

The issue, therefore, is how fast the Filipino algal taxonomists can accept and shift to chemotaxonomy and cytology as fundamental tools toward improvement of our research output.

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ALGAL PRODUCTION AND UTILIZATION RELEVANT TO AQUACULTURE IN THE PHILIPPINES

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INTRODUCTION

Phycological researches in support of aquaculture are a recent development in the Philippines. Progress in this area gained momentum with increased efforts to expand fish farming in the Philippines as a means of producing more animal protein. The Aquaculture Department (AQD) of SEAFDEC, having taken the lead in larval rearing of penaeids and economically important fish species, intensified its search for promising algal species as natural feed. Imported and indigenous algal species were screened and tested for use in hatchery and nursery operations. The vital role of microalgae to sustain growth of larvae during critical stages of development was demonstrated.

This paper presents the researches on algal culture and utilization conducted at AQD, SEAFDEC from 1974 to date. Both brackishwater and freshwater species are covered, with emphasis on freshwater algae.

SELECTED ALGAL SPECIES AS NATURAL FEED

Brackishwater Algae

Diatoms species were considered highly acceptable to *Penaeus monodon* at the early zoea stages. For this reason, early attempts at growing natural feed mentioned unidentified diatoms at 20,000 to 50,000 cells/ml for hatchery operations (AQD Annu. Rept. 1974). *Nitzschia*, *Navicula* and *Thalassiosira* in washings from the seaweed *Sargassum* were also supplied as natural feed in combination with bread yeast.

Mixed diatoms, predominantly *Chaetoceros* sp., were given at 1.5×10^3

cells/ml (AQD Annu. Rept. 1975). A new technique of growing natural feed was applied wherein *Chaetoceros* sp. and *Skeletonema* sp. were grown separate from the larval rearing tank (AQD Annu Rept. 1976). *Skeletonema costatum* imported from Japan had a low temperature requirement which limited its use in the Philippines.

Of the naturally occurring diatom species collected from Buyuan Bay, Iloilo, *Chaetoceros calcitrans* was most promising because of its small size (4-5 μm diam.) and stability in culture under different environmental conditions. This indigenous species has proved very effective as natural food.

More recently, algal species other than those belonging to the Bacillariophyceae have been used as natural feed. *Tetraselmis chunii* and two strains of *Isochrysis galbana* were imported from other laboratories. Local strains of *Tetraselmis* sp. and *Dunaliella* sp. were also tested (AQD Annu. Rept. 1980).

Freshwater Algae

Expansion of the research program to freshwater led to the establishment of the Binangonan Research Station. Here, emphasis on hatchery and nursery operations for tilapia and milkfish necessitated algal production. Algae representing different major groups were selected, namely: *Chlorella ellipsoidea*, Chlorophyta; *Chroococcus dispersus*, Cyanophyta; *Navicula notha*, Chrysophyta; and *Euglena elongata*, Euglenophyta.

The algal composition of "green" water usually varies. The predominant species are *Chlorella* spp. and *Scenedesmus* spp. *Ankistrodesmus* sp. and *Nannochloris* are also present in lesser numbers.

CULTURAL METHODS

Brackishwater

Batch cultures of algae, being the simplest, were used during the early attempts at larval rearing of *P. monodon* (AQD Annu. Rept. 1976). Seawater was enriched with commercial fertilizers (NPK or urea), and naturally occurring algal species were made to "bloom" in rearing tanks. However, problems were encountered when excessive diatom blooms resulted in left-over algae which decayed and polluted the water. Furthermore, fertilizers seemed to be toxic to the larvae. Thus, the procedure was modified so that the algal culture was sand-filtered and the diatom concentrated was pumped into the larval rearing tank (Platon 1978).

More improvements were made later in the algal production system. Stock cultures of different species isolated from Buyuan Bay were main-

tained in the laboratory. *Chaetoceros calcitrans* was among the first algae to be studied extensively and utilized effectively as live, natural food for *P. monodon* larvae. It was established in a culture medium containing macro- and micro-nutrients (Table 1). There were two sources of silicon in the medium, the inorganic salt and "Agrimin" which is a commercially available mixture of micro-nutrients. Growth of *C. calcitrans* in this medium was monitored. When nutrients were replenished daily, growth per day for the first three days was significantly higher (148.7%) than in the control (35.6%) without replenishment (Fig. 1).

Improvements of the basal *Chaetoceros* medium were made later with good results (Platon, 1978). Simplification of culture media for large-scale tank cultures utilized commercial fertilizer (e.g., urea). Silicon was always provided to enhance growth of diatoms.

Table 1. *Chaetoceros* medium

NaNO ₃	- 0.1 g/l
K ₂ HPO ₄	- 1.0 g/l
FeCl ₄	- 0.2 mg/l
Na ₂ SiO ₃	- 0.1 mg/l
Vitamins (B ₁₂ & B ₁)	- 1.0 mg/l
Agrimin*	- 1.0 mg/l
Seawater (boiled/filtered)	- 500 ml
Freshwater	- 500 ml

*Agrimin, a brand name: Manganese, 15%, Boron, 5%; Iron, 8%; Calcium, 3%; Zinc, 10%; Molybdenum, 5-10%; Copper, 5-10%; Potassium, 3%; Silicon, 36%.

In Freshwater

Laboratory cultures. Recent experiments were conducted to compare the growth of selected algal species in three types of media: a) organic, b) inorganic, and c) semi-synthetic (Table 2). Inexpensive organic sources of nutrients such as ipil-ipil (*Leucaena leucocephala*) leaf meal extract and duck manure extract were used. Chemical analyses of the three types of media show some differences in the amounts of major and minor elements required for algal growth (Table 3).

Growth rates of *Chroococcus dispersus* in the different types of media were comparable (Fig. 2). However, the lag phase was longest in the organic medium. It took about eleven days for the logarithmic phase to be reached as compared to only seven days in the inorganic and semi-synthetic media. This may be explained in terms of the slow release of nutrients in the organic medium.

Duncan's Multiple Range Test did not show significant differences among the different media tested for *C. dispersus* (Table 4).

Chlorella ellipsoidea showed the best growth rate in semi-synthetic (K=1.16) and inorganic (K=1.12) media (Table 4). The organic medium was relatively poor for *Chlorella* (Fig. 3).

*Navicula notha** preferred the organic and semi-synthetic media over the inorganic one. With the inorganic medium, the log phase was reached only after 12 days of culture (Fig. 4).

Euglena elongata showed significantly different growth rates based on the type of medium: best in semi-synthetic, moderate in organic, and poor in inorganic (Fig. 5, Table 4).

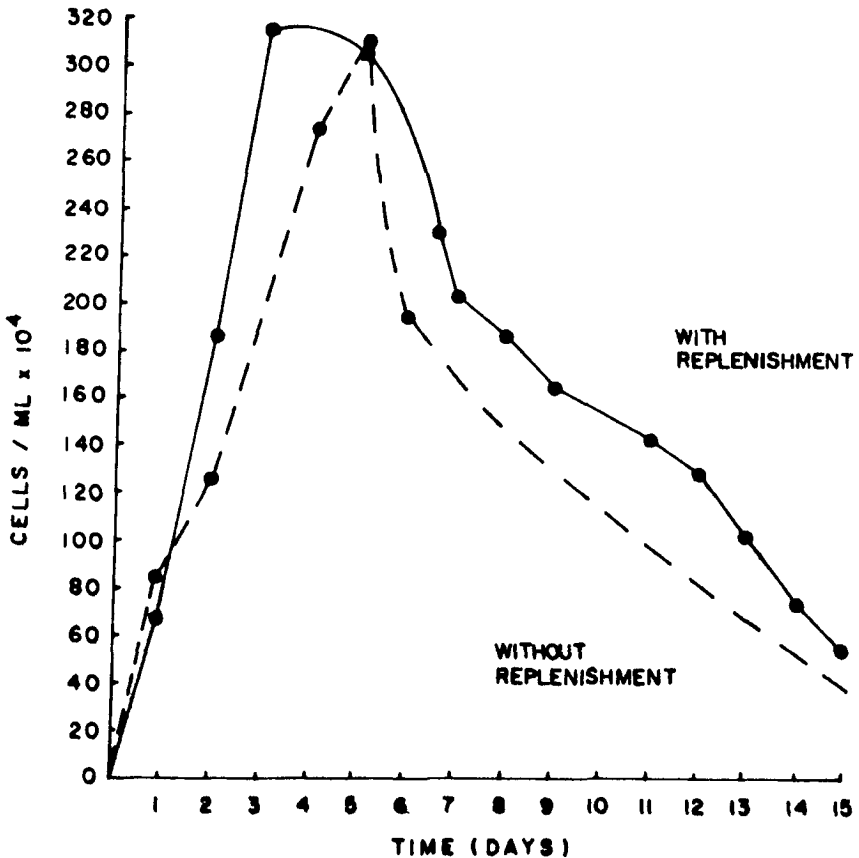


Fig. 1. Growth of *Chaetoceros calcitrans* in *Chaetoceros* medium (see Table 1 for composition).

*Verification of identification courtesy of Dr. Milagrosa Martinez, University of the Philippines at Los Banos.

Table 2. Media for growing selected species of freshwater algae

<i>Inorganic Medium</i>	
	<i>g/l</i>
CaNO ₂	.1258
MgCl ₂	.0664
MgSO ₄	.0450
KCl	.0191
NaCl	.0812
NaHPO ₄	.0229
NaNO ₃	.2573
Na ₂ SiO ₃	.1861
FeCl ₃	.0003
Micronutrients*	1 ml/l

<i>Organic Medium</i>	
Prepare following stocks separately:	<i>ml stock /l</i>
- <i>Ipil-ipil leaf meal extract</i>	
Grind 500 g ipil-ipil leaves; squeeze through cheese-cloth in 500 ml distilled water; autoclave at 20 psi for 15 min.	10
- <i>Duck manure extract</i>	
Pulverize 500 g duck manure; squeeze through cheese-cloth in 500 ml distilled H ₂ O; autoclave at 20 psi for 15 min.	10
- Agrimin** - 10 g/100 ml	1
- Water	159

<i>Semi-synthetic medium</i>	
	<i>ml stock /l</i>
Inorganic medium (without micronutrients)	800
Soil water extract	200
Agrimin**	1

*Composition/100 ml: H₃BO₃, 200 mg; MnCl₂.H₂O, 150 mg; ZnSO₄.7H₂O, 20 mg; CuCl₂.5H₂O, 10 mg; NaMoO₄, 1 mg; Hormex, 1 ml.

**For composition, see footnote for Table 1.

Table 3. Chemical analyses of different media (ppm)

Parameters	Organic	Semi-synthetic	Inorganic
Total inorganic N	.096	.051	.283
Orthophosphate	.00017	.0032	.0153
Silica	20	82.5	91.6
Total Hardness (CaCO ₃)	158	158	-
Calcium	5.58	4.64	30.73
Magnesium	35	35.5	25.5
Sodium	25	180	118.2
Potassium	7.0	28.0	10.02
Manganese	.87	.14	416.4
Iron	.231	.046	.103

Table 4. Mean generation rates (K) of four algae in different media (Figures are means of three replicates)

B. Media	A. Algal Species			
	<i>C. dispersus</i> (A ₁)	<i>C. ellipsoidea</i> (A ₂)	<i>N. notha</i> (A ₃)	<i>E. elongata</i> (A ₄)
Organic media (B ₁)	0.74 ^a	0.85 ^b	0.89 ^a	0.81 ^a
Inorganic media (B ₂)	0.61 ^a	1.12 ^a	0.58 ^b	0.61 ^b
Semi-synthetic (B ₃)	0.65 ^a	1.16 ^a	0.75 ^a	0.87 ^a

Means of the same superscript in a column are not significantly different from one another.

ANOVA for species (A), media (B) and AxB are highly significant. The organic medium exerted the same effect on all the species, i.e., comparable growth rates were shown by the four algal species (Table 5). In the inorganic and semi-synthetic media, *Chlorella* showed significantly faster growth rate. In general, the organic and semi-synthetic media proved best for all the algal species representing different major groups (Table 6).

Based on the foregoing, the possibility of growing selected algae singly or in combination in inexpensive media seems to be a promising alternative in the production of natural food for use in aquaculture.

Outdoor tank cultures. A simplified, continuous culture technique was followed in producing phytoplankton for fry-to-fingerling production. Marine plywood tanks (1,000 liters capacity) were filled with water to a depth of 40 cm only. NPK(14-14-14) was added at 0.1 g/l every three days to sustain algal bloom. Furthermore, one-third of the old culture medium, including algal cells that settled at the bottom, was siphoned out every three days prior to fertilizer application. The same amount of tap water was added as replenishment.

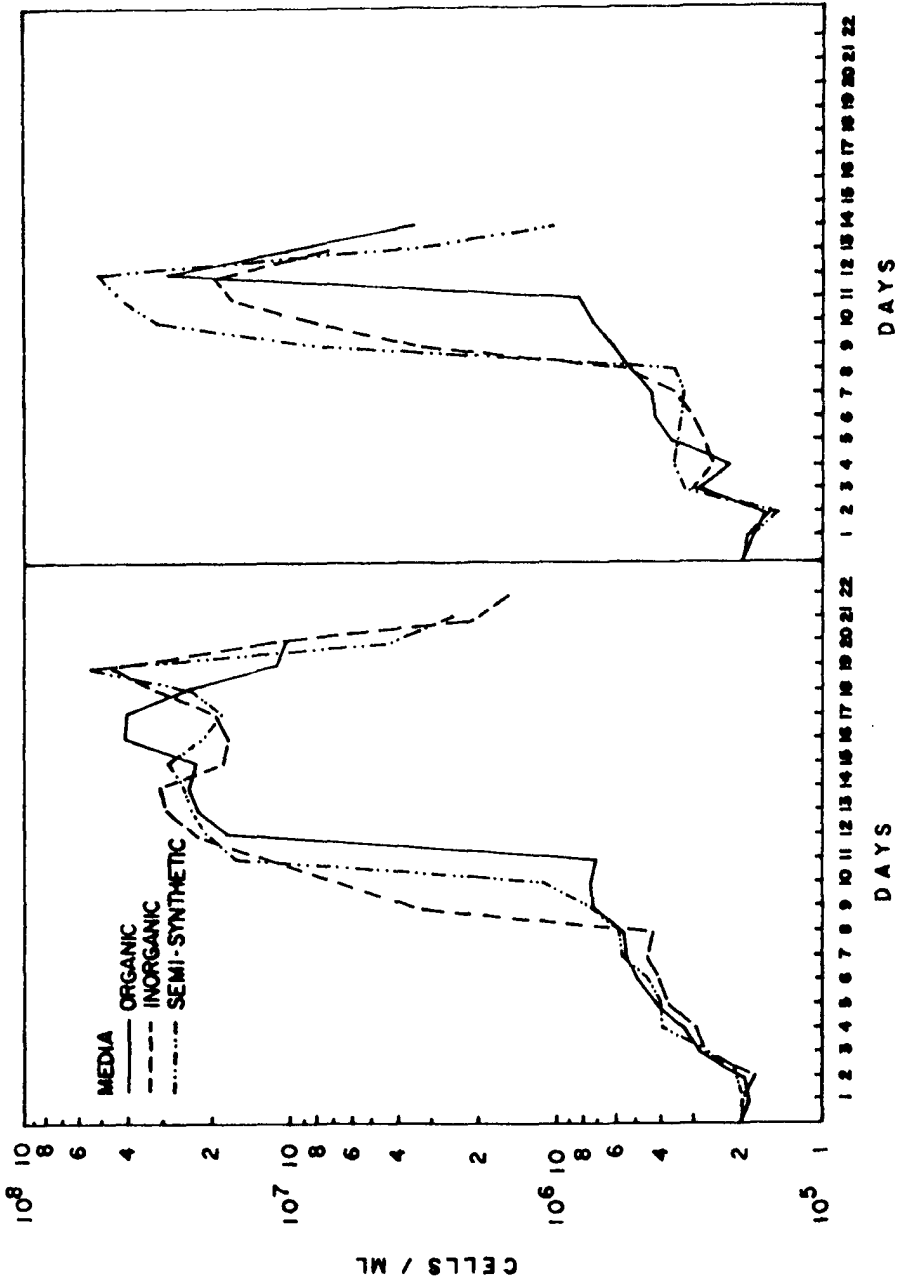


Fig. 2. Growth of *Chroococcus dispersus* in different media.

Fig. 3. Growth of *Chlorella ellipsoidea* in different media.

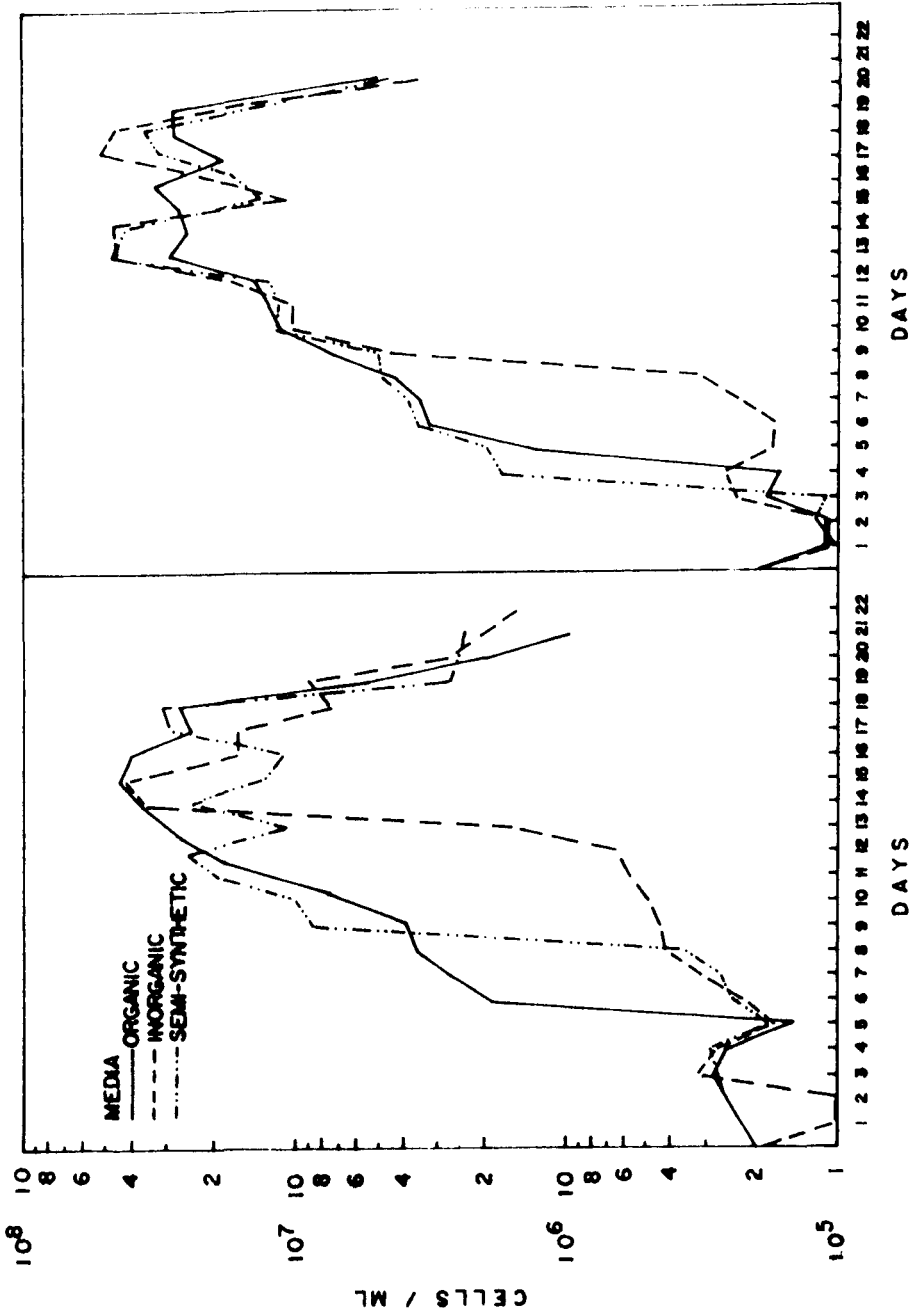


Fig. 4. Growth of *Navicula notha* in different media.

Fig. 5. Growth of *Euglena elongata* in different media.

Table 5. Duncan's Multiple Range tests of growth rates of different algae in different media (Means underlined are not significantly different from one another)

Organic medium			
<i>C. dispersus</i>	<i>E. elongata</i>	<i>C. ellipsoidea</i>	<i>N. notha</i>
<u>.74</u>	<u>.81</u>	<u>.85</u>	<u>.89</u>
Inorganic medium			
<i>N. notha</i>	<i>C. dispersus</i>	<i>E. elongata</i>	<i>C. ellipsoidea</i>
<u>.58</u>	<u>.61</u>	<u>.61</u>	1.12
Semi-synthetic medium			
<i>C. dispersus</i>	<i>N. notha</i>	<i>E. elongata</i>	<i>C. ellipsoidea</i>
<u>.65</u>	<u>.75</u>	<u>.87</u>	1.16

Table 6. Duncan's Multiple Range test for media based on total growth rate means of four species (Means underlined are not significantly different)

Organic (B ₁)	Semi-synthetic (B ₃)	Inorganic (B ₂)
<u>3.29</u>	<u>3.43</u>	2.92

With the method described above, "green" water with a cell density of 150-175 x 10³ cells/ml was produced. This optimum concentration of algal cells can be maintained up to 60 days with proper management.

UTILIZATION OF MICROALGAE IN AQUACULTURE

Larval Rearing of *P. monodon*

One of the major problems in the operation of *P. monodon* hatcheries is the need for a continuous and adequate supply of the right kind of live food. Reports of high fry mortality triggered the all-out effort to conduct extensive feeding studies and screen promising algal species.

Earlier laboratory studies (AQD Annu. Rept. 1976) used live and frozen *Chaetoceros calcitrans* as feed for up to zoea 3 giving as high as 93% and 98% survival, respectively. Thus, there is the possibility of harvesting and storing diatoms for future use to augment the supply of natural feed during times of scarcity. In the same experiment, diatom consumption was determined by monitoring the cell density of the medium with or without larvae. Results showed average diatom consumption per larva at the zoea stages as follows: zoea 1 = 6,000 cells; zoea 2 = 13,100 cells; and zoea 3 = 14,000 cells (Fig. 6).

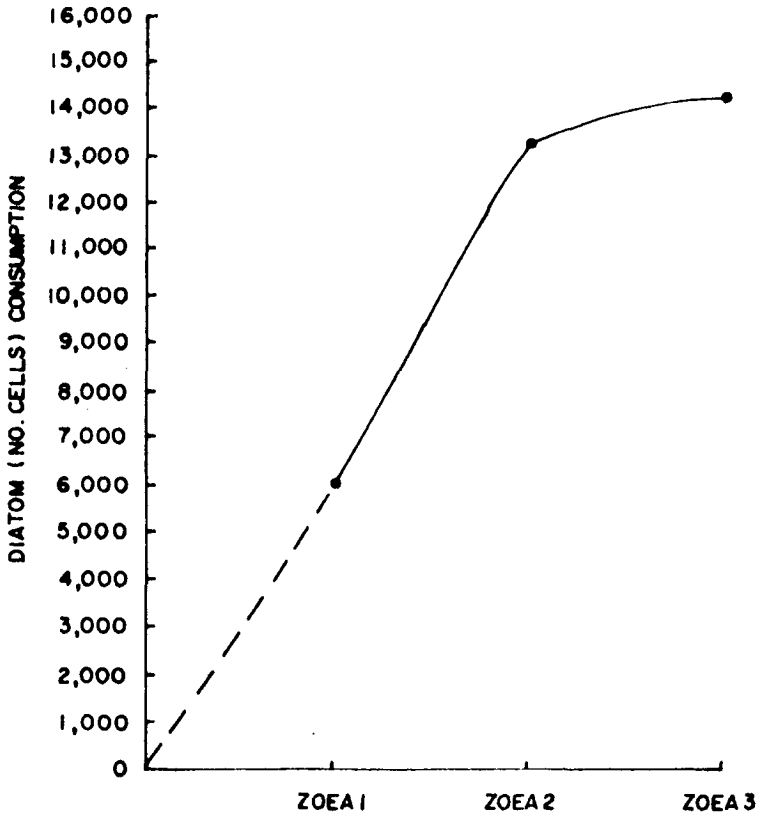


Fig. 6. Estimated algal consumption (*Chaetoceros calcitrans*) of different zoea stages of *P. monodon*.

Later, feeding experiments explored the use of a variety of algal species (AQD Annu. Rept. 1980). Five algal species which include local strains of *Chaetoceros calcitrans* (10×10^4 cells/ml), *Tetraselmis* sp. (5×10^4 cells/ml), *Dunaliella* sp. (5×10^4 cells/ml), two imported strains of *Isochrysis galbana* (7×10^4 cells/ml), and *Skeletonema costatum* (10×10^4 cells/ml) were used as natural feed for *P. monodon* larvae. Highest survival was obtained with *C. calcitrans*.

Sunaz (1980) compared growth and survival of *P. monodon* zoeas given different diatom feeds. Highest mean survival rates were obtained using *Chaetoceros gracilis* (62.90%) and mixed diatoms (60.43%).

Fry to Fingerling Production

Tilapia nilotica. "Green" water consisting of *Nannochloris* sp., *Chlorella* spp. and *Scenedes* spp. was given to *T. nilotica* fry at various concentrations:

a) high density - 150-175 x 10³ cells/ml; b) moderate density - 90-120 x 10³ cells/ml; and c) low density - 50-60 x 10³ cells/ml.

There was a proportionate increase in growth and survival of tilapia fry with increased density of phytoplankton. "High", "moderate" and "low" algal densities gave growth rate of 13.3, 8.9 and 4.7 mg/day, respectively (Table 7). Growth of tilapia fry given limited amounts of phytoplankton was poor and comparable to that given rice bran as feed.

Table 7. Mean weight, survival rate and growth rate of tilapia fry fed phytoplankton at various density levels

Algal density	Weight (g)				Survival rate (%)	Growth rate (g/day)
	0	2nd wk	4th wk	6th wk		
High	0.008	0.164 ^a	0.481 ^a	0.546 ^a	93 ^a	0.0133 ^a
Moderate	0.008	0.098 ^b	0.330 ^b	0.363 ^b	93 ^a	0.0089 ^a
Low	0.008	0.033 ^c	0.099 ^c	0.192 ^c	62 ^b	0.0047 ^c
Rice bran (control)	0.009	0.024 ^c	0.045 ^c	0.186 ^c	36 ^c	0.0045 ^c
<i>F</i> values (ANOVA)	.	23.35*	29.04**	14.40**	-	-

Means with the same superscript in a column are not significantly different from one another (DMRT).

*Significant. **Highly significant.

Gut analysis of *T. nilotica* fry grown in various algal concentrations gave an estimate of the relative intake of algal food at various levels of feeding (Fig. 7). Results showed decreasing algal food in the gut of *T. nilotica* with decreasing amount of phytoplankton in the rearing medium.

Milkfish (Chanos chanos). Stage 1 milkfish fry reared in aquaria with different algal species for five days showed high survival in the *Chlorella-Chroococcus-Euglena* combination and *Oscillatoria* alone (Table 8). These algal species were shown to be suitable natural feeds up to ten days of culture for the *Chlorella-Chroococcus-Euglena* combination and up to 15 days for the treatment *Oscillatoria* alone. Older milkfish fry, 20 days in culture (stage IV), showed poor survival in all treatments.

More experiments are being conducted to pursue the preliminary results described above.

CONCLUSION

Accelerated pace in aquaculture to produce fish protein for the people of Southeast Asia calls for support from all disciplines. Phycology, despite its

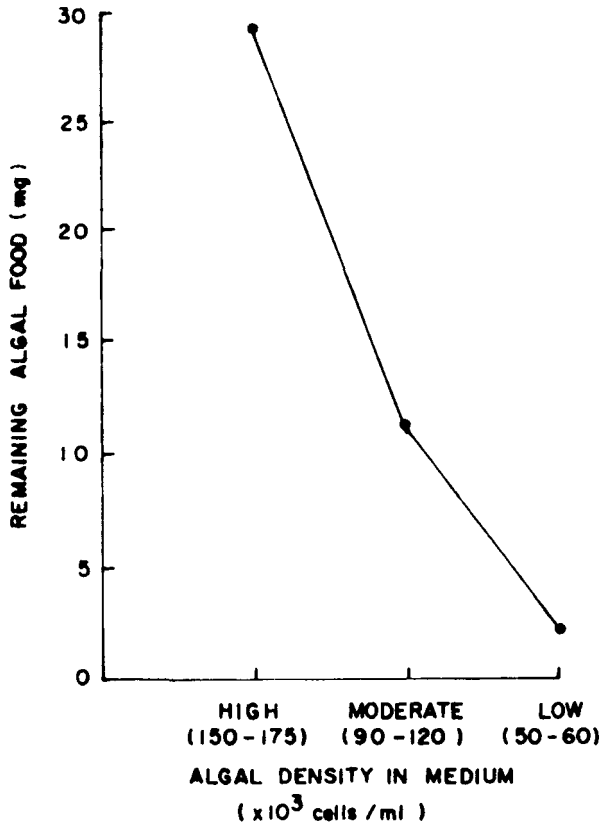


Fig. 7. Remaining algal food after 24 h in gut of *T. nilotica* fingerlings in "green water" with different phytoplankton densities.

Table 8. Mean survival (%) of different stages of milkfish fry given different algal feeds (Figures are averages of three replications)

Algal feed	Stage			
	I (Sept. 3-8)	II (Sept. 9-14)	III (Sept. 16-21)	IV (Sept. 21-27)
<i>Chlorella</i>	41.7	75.0	50.0	8.0
<i>Euglena</i>	75.0	41.7	0	8.0
<i>Oscillatoria</i>	100.0	100.0	100.0	50.0
<i>Navicula</i>	91.7	100.0	75.0	16.7
<i>Chroococcus</i>	41.7	91.7	41.7	8.0
<i>Euglena-Oscillatoria-Navicula</i>	58.0	100.0	66.7	8.0
<i>Chlorella • Chroococcus - Euglena</i>	100.0	100.0	58.0	50.0
Rice Bran	91.7	91.7	66.7	33.0

being a very basic discipline, is most relevant to fish farming. It is in the area of natural food production where the micro-algae have become very important to sustain high fry and fingerling survival.

There is need to integrate efforts in the culture and utilization of algae for greater impact to fisheries development. Manpower and physical resources should be pooled effectively. Only then can we go beyond the laboratory scale and find application in the field.

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